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CENTRAL COUNCIL FOR RESEARCH IN UNANI MEDICINE
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Contents

Editorial

In view of the contemporary global interest generated in Unani medicine, it is important to establish their curative strength through scientific investigations and assure quality and safety through standardization and toxicological studies. Only a scientifically validated Unani medicine can become an integral part of the futuristic medicine and contribute significantly to the ongoing international endeavor against present-day health challenges. Organized research work in this system was, therefore, a need of the hour. In this context, Central Council for Research in Unani Medicine, through its clinical, drug research, literary research, survey & cultivation of medicinal plants programme is contributing significantly for last three decades. *Vitiligo, sinusitis, filariasis, eczema, malaria, infective hepatitis, asthma* are some of the conditions where Unani therapies have earned recognition after scientific validation.

The Council has been publishing the peer reviewed *Hippocratic Journal of Unani Medicine* (HJUM), mainly to bring out fundamental and applied aspects of Unani Medicine. The journal also publishes recent advances in other related sciences and traditional medicines as well as different streams of medical sciences, which have bearing on validation and scientific interpretation of various concpts and strengths of Unani medicine.

In view of an overwhelming response, the journal earlier published twice a year, its periodicity had been changed to quarterly w.e.f. January 2008 to accommodate more articles for quick dissemination of research data among scientific community. The journal has sufficient room for invited articles from luminaries of modern medicine and sciences as well as scholars of Unani medicine. The broad areas being covered include clinical research on single and compound Unani drugs, validation of regimental therapy, Clinical and experimental pharmacological studies, standardization of single and compound drugs, development of standard operating procedures, ethnobotanical studies, experimental studies on medicinal plants and development of agro-techniques thereof, and literary research on classics of Unani medicine. The journal is also open for studies on safety evaluation of Unani and other herbo-mineral drugs, nutraceuticals, cosmotherapeutics, aromatics, oral health, life style disorders, sports medicine etc. and such other newer areas which are the outcome of modern day living.

The current issue of this journal provides 15 original and review papers in the areas of *clinical research, literary and fundamentals of Unani medicine, drug standardization, ethnobotany* and allied disciplines contributed by eminent scholars in their respective fields. It is hoped that data presented will contribute significantly in R&D sector of traditional drugs and prove to be an excellent exposition of current research efforts of scientists in this direction. Council acknowledges the authors for their contributions included in this issue and hope for their continued support in this endeavor. We wish to ensure the readers to bring out the future issues of the journal on time.

We at the CCRUM have been constantly striving to reach to higher standards and make HJUM the leading journal of Unani medicine and related sciences. In this context, we thank our learned reviewers for their invaluable inputs in improving the manuscripts. We sincerely hope and trust that the mission can be accomplished with active partnership of quality-conscious individuals and institutions. Through these lines we seek your cooperation and support in materializing our dreams about the HJUM. In this regard, we request you for your as well as your colleagues' contributions for publication in and subscription to the journal. Further, we will appreciate if the journal is introduced far and wide. We would also welcome esteemed suggestions for achieving the highest standards of quality for the journal.

August 10, 2015

(Prof. Rais-ur-Rahman)
Editor-in-Chief

A Clinical Study to Evaluate the Efficacy of Unani Coded Drugs in Lymphatic Filariasis

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Abstract

The objective of the present study was to evaluate the efficacy of Unani coded drugs combination, UNIM-268 with and without Munzij-Mushil Therapy (M. M. Therapy) in the treatment of lymphatic filariasis, which is caused by the thread-like parasitic filarial worms. 154 selected patients were divided into Group-A (84 patients) and Group-B (70 patients) for their treatment without and with M. M. Therapy respectively. After treatment, the improvements in clinical parameters of disease were observed in the patients of both groups. Filarial oedema volume score was found significantly reduced to 49.08% in Group A patients and 69.68% in Group-B patients. Improvements in lower limb volume in Grade-2 and Grade-3 filarial oedema were found 56% and 44% respectively in Group-A patients; 78.5% and 65.67% respectively in Group-B patients as compared to baseline. Comparatively better response of trial drugs were observed in Group-B patients. Pathological markers such as the level of TLC, total eosinophils counts, A.E.C. and ESR were found significantly reduced after treatment with the coded drugs. Biochemical parameters of Liver function and Kidney function tests were within the normal range after the treatment. Unani coded drugs combination, UNIM 268 was found clinically effective and safe in treatment of lymphatic filariasis.

Keywords: Lymphatic filariasis, Unani coded drugs, UNIM 268, Munzij-Mushil Therapy.

Introduction

Lymphatic filariasis is a vector-borne parasitic disease that is endemic in many tropical and subtropical countries including India. The disease is caused by the thread-like parasitic filarial worms. *Wuchereria bancrofti* is responsible for 90% of the cases; *Brugia malai* and *Brugia timori* are among other species responsible for rest of the 10% cases (Das *et al*., 2002; Fauci *et al*., 2008; Michael *et al*., 1997; Park, 2009). The filarial parasites are transmitted by mosquitoes bites. Preferred habitats of these parasites are the lymphatic vessels and lymph nodes of the hosts, where adult worms (macrofilaria) live for 5-10 years (Subramanian *et al*., 2004).The chronic manifestations including hydrocele and lymphoedema are the result of accumulating infection and worm-induced damage in the lymphatic system. The disease affects over 120 million people worldwide and more than 1.3 billion people in 80 countries are at risk of the infection. About 43 million people are suffering with chronic lymphoedema and hydrocele (Michael *et al*., 1996; Michael *et al*., 1997; WHO, 2012). India contributes about 40% of

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the total global burden and accounts for about 50% of population at risk of filarial infection (Ramaiah *et al.*, 2000; Zagaria *et al.*, 2002). Bihar has the highest endemicity (over 17%) followed by Kerala (15.7%) and Uttar Pradesh (14.6%) etc.

According to Unani scholars, the lymphatic filariasis (Da'ul Feel) is swelling of feet and calf. Affected leg becomes hugely swollen in advanced stage similar to leg of an elephant, therefore, it is termed Da'ul Feel (Kabiruddin, 2007). According to Abu Baker Mohammad Bin Zakariya Razi and Ibn al-Quff Masihi, the disease is caused by the Black Bile (Sawda) (Razi, 1962; Masihi, 1356H), while Nuh Qamari has mentioned the abnormal flow of the thick matters towards the legs as causative factor of the disease (Qamari, 2008). Some Unani physicians say that disease is due to the abnormality of phelgm and Black Bile (Antkari, 2009), while few add pure sanguine temperament to the list (Khan, 1885).

Diethyl carbamazine (DEC) is the only drug available for therapeutic control of lymphatic filariasis (Park, 2009). Due to serious allergic reaction and others adverse effect of the available drugs in modern medicine, it is need of time to find out efficacious and safer drugs for treatment of lymphatic filariasis. Unani medicine is one of them; this system not only provides the drugs information in abundance but also claims that the drugs have least or no adverse effect on human body.

Unani physicians, since ancient times, have mentioned single and compound formulation for treatment of lymphatic filariasis but clinical data is not available therefore this study was conducted with aim to assess the clinical efficacy of Unani coded drugs in the treatment of lymphatic filariasis.

Material and Methods

Study Drug

The study drug was coded drugs UNIM 268 with UNIM 270, UNIM 271 and UNIM 272 used with and without Munzij-Mushil therapy.

Study Design

The study was designed as double blind open clinical trial.

Patients Selection

Diagnosis of each case was made with the help of detailed history in respect of the patients i.e. history and physical examination, allergic history and others systemic examinations as well as the laboratory investigations. The patients

having lower limb lymphoedema and presenting one or more signs and symptoms of lymphatic filariasis, such as fever with and without rigors, lymphadenitis, lymphangitis, lymphoedema, dermatosclerosis, headache, malaise, utricaria, orchitis and chyluria were screened for the clinical evidences of the disease. All the screened patients with confirmed clinical diagnosis, who met the inclusion/exclusion criteria, were selected for the study.

Inclusion Criteria

- Patients of either sex in the aged group of 11-65 years.
- Patients having lower limb lymphoedema.
- Patients with presenting symptoms of the lymphatic filariasis.
- Patients willing to sign informed consent form to participate in the study.
- Patients willing to comply with various demands of study.

Exclusion Criteria

- Patients below 11 years and above 65 years of age.
- Patients, who were suffering from others diseases.
- Patients with HB% less than 50%.
- Malnutrition patients.
- Pregnant or lactating women.
- Patients willing to take Unani therapy in writing.

Treatment of Patients

The present double blind open clinical study was approved by the institutional ethical committee. 154 patients of lymphatic filariasis having lower limb lymphoedema, were selected for the study at Regional Research Institute of Unani Medicine, Patna between April 2008 and March 2014. Patients were divided into two groups i.e. Group-A (84 patients) and Group-B (70 patients) and were treated with coded drugs combinations UNIM 268 used with and without M.M. Therapy, mentioned as below;

Group-A patients (Treatment without M.M. Therapy)

UNIM-268, two tablets of 500 mg twice daily on empty stomach for 90 days,

UNIM-270, five grams powder,

UNIM-272, 20 ml liquid,

UNIM-270 and UNIM-272 were mixed together and applied on the affected part at night for 90 days.

UNIM-271, 20 grams of coarse powder boiled in one litre of water was slowly dropped (Nutool) on the affected part daily for 10-15 minutes before applying paste at night.

Group-B patients (Treatment with M.M. Therapy)

(i) UNIM-MUNB (Munzij)

Decoction of UNIM – MUNB (35 gm of coded drugs) in the dose of 125 ml was given orally once a day on empty stomach in the morning for 10 to 15 days according to chronicity of disease or till the appearance of Nuzj in the urine.

(ii) UNIM-MUSB (Mushil)

35 gm of UNIM (MUSB) was added to 15 gm UNIM (MUNB) recipe and decoction of UNIM-MUSB (coded drugs) in dose of 125 ml was given orally in night for 5 days on alternate days. The treatment with UNIM-MUSB was started after end of treatment with UNIM-MUNB.

(iii) UNIM-TAB (Tabreed) (coded drugs)

Infusion of UNIM-TAB (crude drugs) in the dose of 50 ml was given orally on empty stomach on alternate days to UNIM-MUSB (Mushil) administration for 5 days.

After Munzij Mushil Therapy, Group-B patients were given the same treatment as was given to Group-A patients.

Treatment Duration

Treatment duration for Group-A patients was 90 days and for Group-B patients, it was 90 days in addition to numbers of days of M.M. Therapy.

Clinical Evaluation

The effects of trial drugs were assessed on the clinical parameters of the lymphatic filariasis. The severity of lymphodema was measured on 4 pointer scale in terms of lower limb oedema volume i.e. no oedema (absent=0), Grade-1 (mild oedema=1), Grade-2 (moderate oedema=2) and Grade-3 (severe oedema=3) for appropriate assessment and statistical evaluation of the trial drugs. The patients were clinically examined at the baseline, after M.M. Therapy, at regular interval of 30 days and after the treatment. They were asked about the improvement or worsening in their symptoms during the course of the study.

Safety Assessment

The safety was assessed by monitoring adverse events either volunteered by the patients or elicited by the investigator by clinical as well as laboratory investigations at the baseline, after M.M. Therapy, at regular interval of 30 days and after the treatment. The laboratory tests included Haemological Test (TLC, DLC, ESR), Liver Function Test (serum bilirubin, SGOT, SGPT, alkaline phosphatase) and Kidney Function Test (blood urea, serum- creatinine).

Statistical Analysis

All data were statistically analyzed by applying 't' test. Probability level of less than 5% was considered as statistically significant.

Results

Mean age of the selected patients was 38.32 years. The distribution of characteristics / demographic data of patients in accordance with their age and gender; dietary habits and temperaments; chronicities & status of the disease and socio economic status are summarized in Tables 1-4, respectively.

The effects of trial drugs on the clinical parameters of lymphatic filarisis are depicted in table-5. After treatment, scores of clinical parameters of the disease including fever with and without rigors, lymphadenitis, lymphangitis, dermatosclerosis, headache, malaise, utricaria and orchitis were found subsided in patients of both groups.

Table 1: Distribution of patients according to age and sex.

Total No. of cases- 154

Age groups (in years)	Male		Female		Total	
	No.	% age	No.	% age	No.	% age
11-20	06	03.90	10	06.49	16	10.39
21-30	09	05.84	22	14.29	31	20.13
31-40	16	10.39	34	22.08	50	32.47
41-50	10	06.49	12	07.78	22	14.27
51-60	20	12.98	14	09.09	34	22.07
61-65	1	00.65	0	00.00	01	00.65
Total	62	40.26	92	49.74	154	100

Table 2: Distribution according to dietary habits and Temperament of the patients.

Temperament of patients	Dietary habits				Total	
	Veg.		Non-Veg.		No.	Percentage
	No.	Percentage	No.	Percentage		
Damvi (Sanguine)	03	01.95	01	00.65	04	02.60
Balghami (Phlegmatic)	23	14.93	101	65.584	124	80.52
Safravi (Bilious)	03	01.95	14	09.09	17	11.04
Saudavi (Melancholic)	03	01.95	06	03.89	09	05.84
Total	32	20.78	122	79.22	154	100

Table 3: Distribution according to chronicity and status of the disease

Chronicity of disease	Status of disease				Total	
	New status		Known status		No.	Percentage
	No.	Percentage	No.	Percentage		
Up to 1 Year	48	31.17	07	04.54	55	35.71
01-03 Years	16	10.39	56	36.36	72	46.75
03-05 Years	06	03.90	18	11.69	24	15.59
Above 5 Years	02	01.30	01	00.65	03	01.95
Total	72	46.75	82	53.25	154	100

Table 4: Socio – Economic status of the patients.

Socio Economic Status	No. of Patients	Percentage
Lower Income Group	99	64.28
Middle Income Group	52	33.77
Higher Income Group	03	01.95
Total	154	100

Table 5: Clinical parameters of lymphatic filariasis before and after treatment

Clinical parameters	Group A (84 patients)			Group B (70 patients)		
	Base Line	After Treat-ment	Per-centage reduction	Base Line Line	After Treat-ment	Per-centage reduction
Fever with Rigors	49	4	91.8	38	1	97.3
Fever without Rigors	16	1	93.7	11	0	100
Lymphadenitis	77	10	87.0	68	8	88.2
Lymphangitis	77	9	88.3	68	12	82.3
Dermato-sclerosis	3	0	100	4	1	75.0
Headache	35	4	88.6	17	2	88.2
Malaise	30	6	80.0	13	0	100
Urticaria	3	1	66.7	3	0	100
Orchitis	4	0	100	3	0	100
Chyluria	5	0	100	8	0	100

The difference in mean age of Group-A and Group-B patients and effects of trial drugs on filarial oedema volume are depicted in table-6. Mean age of the patients placed in Group-A and Group-B were 38.30 ± 1.28 and 38.27 ± 1.69 respectively. The difference in mean age of the patients in Group-A and Group-B was insignificant ($p>0.05$).

After treatment, mean scores of lower limb oedema volume in Group-A patients, was found reduced from 2.18 ± 0.07 to 1.11 ± 0.07, while in Group-B patients, scores was reduced from 2.54 ± 0.06 to 0.77 ± 0.08 as compared to base line. Percentage reduction in mean scores of oedema volume in Group-A and Group-B patients were found 49.08% ($p<0.001$) and 69.68% ($p<0.001$) respectively as compared to baseline (table-6).

After treatment, mean scores of lower limb volume in Grade-2 and de-3 filarial oedema of Group-A patients were found decreased from 2.0 ± 0.0 to 0.88 ± 0.08 and 3.0 ± 0.0 to 1.68 ± 0.16 respectively, while mean scores of grade-2 and grade-3 filarial oedema of Group B patients were found decreased from 2.0 ± 0.0 to 0.43 ± 0.09 and 3.0 ± 0.0 to 1.03 ± 0.10 respectively as compared to

Table 6: Comparison of mean age of patients and lymphoedema scores before and after the treatment.

		Treatment Groups	
		Group A (84 patients)	Group B (70 patients)
		Mean ± S.E.M	Mean ± S.E.M
Age of patients (Years)		38.30 ± 1.28	38.27 ± 1.69 (N.S.)
Day of measurements	Base Line	2.18 ± 0.07	2.54 ± 0.06
	After Treatment	1.11 ± 0.08***	0.77 ± 0.08***
Percentage reduction		49.08%	69.69%

Statistical analysis by 't' test. ***p<0.001 (Highly significant), p>0.05 (N.S.) as compared to baseline;

Table 7: Mean percentage reduction of limb volumes in different grades of filarial oedema.

Lympho-edema Grades	Treatment Groups							
	Group-A (84 patients)				Group-B (70 patients)			
	Mean ± S.E.M			%age reduction	Mean ± S.E.M			%age reduction
	Nos	Base-line	After Treatment		Nos.	Base-line	After Treatment	
Grade-3	25	3.0±0.0	1.68 ±0.16***	44.00	39	3.0±0.0	1.03 ±0.10***	65.67
Grade-2	49	2.0±0.0	.88 ±0.08***	56.00	30	2.0±0.0	0.43 ±0.09***	78.50
Grade-1	10	1.0±0.0	0.80±0.4	20.00	1	1	1	0.0

Paired 't' test, ***p<0.001(Highly Significant) as compared to baseline.

baseline. Percentage improvement in grade-2 and grade-3 filarial oedema were 56% (p<0.001) and 44% (p<0.001) respectively in Group A patients; 78.5% (p<0.001) and 65.57% (p<0.001) respectively in Group B patients as compared to baseline (table-7).

At the end of the study, percentage reduction in total leucocytes counts (TLC), total eosinophil counts%, absolute eosinophil counts (A.E.C.) and erythrocyte

Table 8: Effects of Unani coded drugs combination, UNIM 268 with and without M.M. Therapy on the levels of total Eosinophil counts, Erythrocyte Sedimentation Rate (E.S.R.), Absolute Eosinophil Counts (A.E.C.) and Total Leucocytes Counts (T.L.C.) before and after treatment of lymphatic filariasis patients.

Haematological parameters	Treatment Groups					
	Group-A			Group-B		
	Mean ± S.E.M		%age reduction	Mean ± S.E.M		%age reduction
	Base-line	After Treatment		Base-line	After Treatment	
Total Eosinophil counts%	6.39 ± 0.28	4.37 ± 0.26***	31.61	7.10 ± 0.46	4.47 ± 0.21***	37.06
E.S.R. (mm/hr)	21.70 ± 2.15	10.75 ± 0.81***	50.46	26.80 ± 3.87	14.95 ± 2.16***	44.21
A.E.C. (cell/cu mm)	417.19 ± 19.10	282.07 ± 16.90***	32.38	456.98 ± 25.87	302.26 ± 17.86***	33.86
T.L.C. (1000/Cu mm)	6756.63 ± 147.32	6425.98 ± 81.06*	4.89	7015.31 ± 208.54	6589.51 ± 155.00*	6.07

*p<0.05(Significant), ***p<0.001(Highly Significant) as compared to baseline.

sedimentation rate (ESR) in Group A patients were found 4.89% (p<0.05), 31.61% (p<0.001), 32.38% (p<0.001) and 50.46% (p<0.001) respectively, while the percentage reduction in Group B patients were found 6.07% (p<0.01), 37.04% (p<0.001), 33.86% (p<0.001) and 44.21% (p<0.001) as compared to baseline (table-7).

Biochemical parameters of Liver Function Test (S. Bilirubin, SGOT, SGPT, Alk.Phosphatase) and Kidney Function Test (Blood Urea, S.Creatinine), as assessed by laboratory investigation were found within normal range after treatment with trial drugs.

Discussion

The present study reveals that incidences of the disease increase with age with the highest incidence of 32.47% in young age group 31-40 years. After age of 40 years, the incidences of the disease start decreasing with age with the lowest incidence of 0.65% after 60 years (table-1). The highest incidence of the disease in young age group 31-40 years may be due to involvement of most of the people of this age-group in rice farming, fishing and others outdoor activities, where man-

mosquitoes contact rate increases. This study is in accordance with the finding that filarial infection increased steadily with age reaching maximum in age-group 30-40 years (Steel *et al.*, 2002).

The present study disclosed the prevalence of the disease was highest 80.52% among the people of phlegnatic (Balghami) temperament (table-2). It is in consonance with the observation that disease is due to the abnormality of phlegm (Antaki, 2009).

The study indicates that 53.25% cases were switched-over from other systems of medicines to Unani system of medicine (table-3). This suggests that patients not getting relief in other systems of medicine came for treatment of filarial oedema with Unani system of medicine. The present study further reveals that 46.75% patients had chronicities of disease from 1 to 3 years. It may be due to the lack of awareness of the disease amongst the most of the people, who left the filarial infections untreated for long period till it becomes chronic.

The highest incidence of the disease 64.28% was observed in patients of low income group (table-4) because poor sanitary conditions associated with low socio-economic status of the community, make the environment conductive for breeding of vector mosquitoes that facilitate the transmission of the disease by mosquitoes. The poor people living in very remote areas also lack access to health care and are not aware of the disease therefore prevalence of the disease was found in low income group.

Clinical parameters of lymphatic filariasis including fever with and without rigors, lymphadenitis, lymphangitis, dermatosclerosis, headache, malaise, utricaria and orchitis were found subsided after treatment with the trial drugs but comparatively better response of coded drugs was observed in improvements of the clinical parameters of group B patients. (table-5).

The present study has disclosed and exhibited that mean scores of filarial oedema volume, which is the main cause of social suffering, was found significantly reduced to 49.08% ($p<0.001$) and 69.69% ($p<0.001$) in Group-A and Group-B patients respectively as compared to baseline (table-6). Percentage improvements in lover limb volume in Grade-2 and Grade-3 filarial oedema of Group-A patients were found 56% ($p<0.001$) and 44% ($p<0.001$) respectively, while percentage improvements in grade-2 and grade-3 oedema cases of Group-B patients were found 78.5% ($p<0.001$) and 65.57% ($p<0.001$) respectively as compared to baseline (table-7). This shows that trial drugs were found very effective in reducing the lower limb oedema volume, when used with the M.M. therapy. This finding is in accordance with the observation of Razi that decrease in volume of the affected leg can be achieved through purgation of the disease (Razi, 1962).

The present study reports that significant improvements were observed in haematological parameters, such as total leucocytes counts (TLC) ($p<0.05$), total eosinophil counts ($p<0.001$), absolute eosinophil counts (A.E.C.) ($p<0.001$) and erythrocyte sedimentation rate (ESR) ($p<0.001$) in the patients of both groups after treatment with trial drugs (table-8). It is well known that absolute eosinophil counts, which was increased in the case of lymphatic filariasis (Andrea et al.,2004), was found significantly decreased after treatment with the unani coded drugs. The ESR that indirectly measures the presence of inflammation in the body and reflects the tendency of red blood cells to settle more rapidly in the case of some disease status was found significantly reduced after the treatment with trial drugs.

All safety parameters including biochemical parameters of Liver Function Test (S. Bilirubin, SGOT, SGPT, Alk.Phosphatase) and Kidney Function Test (Blood Urea, S.Creatinine) were found within normal range before and after the treatment. This confirms that trial drugs are safe for treatment of lymphatic filariasis.

Conclusion

On the basis of above observations, it can be concluded that the coded drugs combination UNIM 268 with and without Munzij-Mushil Therapy is clinically very effective and safe in the treatment of lymphatic filariasis. Lower limb oedema volume was markedly reduced, when the coded drugs were used with Munzij-Mushil Therapy. The trial drugs can safely be prescribed to the patients for the management of lymphatic filariasis.

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Therapeutic Evaluation of Unani Coded Drug UNIM- 855 in Tooth Hypersensitivity

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Abstract

The therapeutic efficacy of Unani coded drug UNIM- 855 was evaluated in 30 patients of tooth hypersensitivity of either sex ranging in age from 20 to 60 years. 2 gm of the test drug as fine powder was advised to be rubbed over the surface of the gums and teeth by the index finger and left for five minutes then rinse with fresh water. Scoring for tooth hypersensitivity was done before treatment and at weekly intervals after initiating the treatment. Treatment was given initially for 15 days and extended up to 60 days if required. Out of 30 cases, 12 got 100% response, 10 got 71-99% response, 2 got 51-70% response, 4 got 31-50% response and 2 got no response. Thus the Unani coded drug UNIM- 855 is shown to be quite effective in reducing the tooth hypersensitivity.

Keywords: Tooth hypersensitivity, Unani Medicine, UNIM-855

Introduction

It is not unusual for patients to complain of root surface sensitivity, which is annoying sharp pain usually associated with gingival recession and exposed root surface. Several theories have been advanced to explain the unusual sensitivity and response of such exposed dentin to a stimulus or irritation. The most accepted theory is the hydrodynamic theory, which postulates that the pain results from indirect innervations caused by dentinal fluid movement predentin. Dentinal hypersensitivity is a particular problem in patients immediately after periodontal surgery. A number of treatments have been used to provide relief, such as topical fluorides, oxalates solutions, dentin bonding agents and desensitizing tooth pastes. But on prolong use of these drugs may have its own side effects as well as limited efficacy while Unani drugs do not have such side effects , so it is worthwhile to test the Unani drugs on scientific parameters (Bal and Kundalgurkhe, 1999; Curro, 1990; Orchardson and Collins, 1987).

Dental hypersensitivity is not elaborately discussed in Unani classics (Razi, 1998). It is described in Al-Mualijat Buqratia as “this is gaseous disease which induces an acute condition on dental surrounding resulting accumulation of gases and pain” (Tabri, 1997); particularly this condition is associated with tinismus appearance in the nerves. A number of Unani drugs are reported to be effective in dental hypersensitivity by many eminent Unani physicians (Khan, 1902). Hence a clinical study was planned to evaluate the therapeutic efficacy of Unani formulation in the patients of tooth hypersensitivity.

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Material and Methods

Type of trial: An open-label clinical study.

Subject selection

Patients attending the dental OPD of CRIUM, Lucknow for treatment of tooth hypersensitivity were screened for inclusion and exclusion criteria. They have been informed about the nature and objective of the trial before enrolling them.

Treatment schedule

Drug: UNIM-855

Dosage and mode of administration

2 g. coded drug was given to the patient and advised to apply over the sensitive teeth with gentle rubbing with index finger for five minutes in the morning and at bed time daily and left for five minutes, to be later rinsed off with fresh water.

Follow up method and interval during treatment

The patients were instructed to visit every week for assessment of tooth hypersensitivity and recording was done accordingly.

Criteria of assessment

Tooth hypersensitivity was observed by subjective scale from 0-100. Initially tooth hypersensitivity was considered as 100%. After subsequent visit, reduction of tooth hypersensitivity was recorded in terms of percentage according to patient's spontaneous report on pro forma at interval of a week.

Determination of percent improvement in each case was categorized in following groups:

1. 100% improvement
2. 71-99% improvement
3. 51-70% improvement
4. 31-50% improvement
5. <30% improvement
6. 0% improvement

Grading of the disease

Grade I: Subject responds to air stimulus but does not request discontinuation of stimulus.

Grade II: Subject responds to air stimulus and requests discontinuation or moves from stimulus.

GradeIII: Subject responds to air stimulus, considers stimulus to be painful, and requests discontinuation of the stimulus.

Data recording: Data recording was done on separate case sheet for each subject as base line and every follow up i.e. is every week up to two months.

Observations

The drug UNIM 855 was tried in 30 patients of tooth hypersensitivity of either sex in age group of 20 to 60 years.

The overall response was noted as 100% in 12 cases, 71-99% in 10 cases, 51-70% in 2 cases, 31-50% in 4 cases and no response was in 2 cases. (Table 1)

Highest number (13 cases) of 100% response was seen in the age group of 20 to 30 years, 5 cases in the age group of 31-40 years, 8 cases in the age group 41-50 years and 4 cases were in the age group of 50-60 years (Table 2).

Out of 30 cases 57% were male and 43% were female. Excellent response (100%) was noted in 2 cases of female and 10 cases of male (Table 3).

Table 1: Overall response

100%	71-99%	51-70%	31-50%	Below 30%	No response	Total (%)
12(40.00)	10(33.33)	2(6.66)	4(13.33)	—	2(6.66)	30(100.0)

Table 2: Age-wise response

Age in year	Response						Total (%)
	100%	71-99%	51-70%	31-50%	Below 30%	No response	
20-30	5	3	1	2	-	2	13(43.33)
31-40	3	—	1	1	-	-	5(16.66)
41-50	3	5	-	-	-	-	8(26.66)
51-60	1	2	-	1	-	-	4(13.33)
Total (%)	12(40.00)	10(33.33)	2(6.66)	4(13.33)	-	2(6.66)	30(100.00)

Table 3: Sex-wise response

Sex	Response						Total (%)
	100%	71-99%	51-70%	31-50%	Below 30%	No response	
Male	10	5	-	1	-	1	17(56.66)
Female	2	5	2	3	-	1	13(43.33)
Total (%)	12(40.00)	10(33.33)	2(6.66)	4(13.33)	-	2(6.66)	30(100.00)

According to temperament wise response 21 cases were balghami temperament out of which 5 cases got (100%) response, 9 cases got 71-99%, 2 cases got 51-70%, 4 cases got 31-50% and 1 case got no response. 6 cases were damvi temperament, out of which 5 cases got 100% response and 1 cases got no response. 2 cases were safravi temperament which got 100% response. 1 case was Saudavi temperament which got 71-99% response (Table 4).

As per grade wise response, maximum number (7 cases) of 100% response was seen in grade I (Table 5).

Table 4: Temperament-wise response

Temperament	Response						Total (%)
	100%	71-99%	51-70%	31-50%	Below 30%	No response	
Balghami	5	9	2	4	-	1	21(70.00)
Damvi	5	-	-	-	-	1	6(20.00)
Safravi	2	-	-	-	-	-	2(6.66)
Saudavi	-	1	-	-	-	-	1(3.33)
Total (%)	12(40.00)	10(33.33)	2(6.66)	4(13.33)	-	2(6.66)	30(100.00)

Table 5: Grade-wise response

Grade of disease	Response						Total (%)
	100%	71-99%	51-70%	31-50%	Below 30%	No response	
Grade-I	7	3	1	2	-	1	9(30.00)
Grade- II	3	3	-	1	-	-	7(23.33)
Grade-III	2	4	1	2	-	1	14(46.66)
Total (%)	12(40.00)	10(33.33)	2(6.66)	4(13.33)	-	2(6.-66)	30(100.00)

In terms of duration of treatment, the maximum response i.e. 23 (76.67%) cases were noted in 16 to 30 days of treatment out of which 11 cases got 100% response, 7 cases got 71-99%, 1 case got 51-70%, 3 cases got 31-50% and 1 case got no response. Minimum response that is 1 case (3.33%) was noted in up to 15 days of (Table 6).

According to chronicity, the maximum number of cases i.e. 26(86.66%) were in less than 1 year chronicity out of which 11 cases got 100% response, 7 cases got 71-99%, 2 cases got 51-70%, 4 cases got 31-50% and no response was observed in 2 cases (Table 7).

Dietary habit wise response showed that 100% response observed equally in vegetarian and non-vegetarian (Table 8).

Table 9 indicates that out of 30 subjects 22 subjects were gutka/tobacco chewer and 8 subjects were gutka/tobacco nonchewerwhich shows that gutka/ tobaccochewing has important role in developing tooth hypersensitivity. In chewing group, 100% and 71-99% response was noted in 8 cases while 2cases

Table 6: Duration of treatment-wise response

Duration of treatment in days	Response						Total (%)
	100%	71-99%	51-70%	31-50%	Below 30%	No response	
Up to 15 days	-	-	1	-	-	-	1(3.33)
16-30	11	7	1	3	-	1	23(76.67)
31-45	1	3	-	1	-	1	6(20.00)
Total (%)	12(40.00)	10(33.33)	2(6.66)	4(13.33)	-	2(6.66)	30(100.00)

Table-7: Chronicity-wise response

Chronicity (In days)	Response						Total (%)
	100%	71-99%	51-70%	31-50%	Below 30%	No response	
Less than 1 year	11	7	2	4	-	2	26(86.66)
01-03	-	2	-	-	-	-	2(6.66)
04-06	1	1	-	-	-	-	2(6.66)
Total (%)	12(40.00)	10(33.33)	2(6.66)	4(13.33)	-	2(6.66)	30(100.00)

Table 8: Dietary habit-wise response

Dietary habit	Response						Total (%)
	100%	71-99%	51-70%	31-50%	Below 30%	No response	
Vegetarian	6	4	2	4	-	1	17(56.66)
Non Vegetarian	6	6	-	-	-	1	13(43.33)
Total (%)	12(40.00)	10(33.33)	2(6.66)	4(13.33)	-	2(6.66)	30(100.00)

Table 9: Oral hygiene habit-wise response

Oral hygiene habit	Response						Total (%)
	100%	71-99%	51-70%	31-50%	Below 30%	No response	
Gutka/ Tobacco chewer	8	8	2	3	-	1	22(73.33)
Gutka/ Tobacco non-chewer	4	2	-	1	-	1	08(26.67)
Total (%)	12(40.00)	10(33.33)	2(6.66)	4(13.33)	-	2(6.66)	30(100.00)

got 51-70%, 3 cases got 31-50% and 1 case got no response.In nonchewing group, 100% response was observed in 4 cases while 2 cases got 71-90%, 1 case got 31-50% and 1 case got no response.

Results and Discussion

30 patients suffering from tooth hypersensitivity were treated with Unani coded drug UNIM-855 for a period of 15 to 60 days. Out of 30 cases 100% response was noted in 12 cases, 71-99% in 10 cases, 51-70% in 2 cases, 31-50% in 4 cases and no response noticed in 2 cases. So we may conclude that Unani coded drug UNIM-855 is quite effective in reducing tooth hypersensitivity.

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An Aetiological and Preventive Review on *Isqate Janeen* or *Isqate Hamal* (Abortion)

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Abstract

Abortion is the ending of pregnancy by expulsion of the fetus or embryo from the womb before reaching the viability to survive. It is a sensitive and very distressing, sad situation for the expecting mother and is also contentious issue with religious, moral, cultural and political dimensions. In Unani System of Medicine it is known as *"Isqate Hamal or Isqate Janeen"*. It is the commonest problem in obstetrics, which is studied from the early era of medicine till now. Almost every Unani physician has described about the causes and preventive measures of abortion. They were mainly focused on prevention of abortion by various non-pharmacological and pharmacological methods. Non-pharmacological methods like bed rest, avoidance of jerky movements etc; whereas pharmacological methods include systemic use of astringent drugs both single and compound formulations as well as local applications, which are very effective in the management of abortion are recommended. The literature of Unani medicine has wealth of treasure on abortion, but the issue is not in a systemic way. Hence, in this review scattered knowledge and description about the abortion and its consequences has been systematized in an easy way. An attempt has been made to systematically review the literature on abortion and to make the reader familiar with the contributions of Unani physicians.

Keywords: *Isqate Hamal, Isqate Janeen*, Aetio-pathogenesis, Prevention, Unani medicine.

Introduction

Abortion is a sensitive and contentious issue with religious, moral, cultural, and political dimensions. It is also a public health concern in many parts of the world. The World Health Organization (WHO) estimates that worldwide 210 million women become pregnant each year and that about two-thirds of them or approximately 130 million deliver live infants, the remaining one third of pregnancies end in miscarriage, stillbirth or in induced abortion (World Health Organization, 2011; Katz, 2012; Simpson and Jauniaux, 2010)

Abortion is the ending of pregnancy by the removal of a fetus or embryo before it is able to survive from the womb. (Susan Storck, 2011) An abortion can occur spontaneously, in which case it is often called a miscarriage. It can also be purposely caused in which case it is known as an induced abortion. Miscarriage is the natural death of an embryo or fetus in the womb. It takes place in the early stages of development of the fetal viability. Miscarriage may occur for many reasons, all of which cannot be identified. (National Coordinating Centre for

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Women's and Children's Health, UK (2013). Some of these causes include genetic, maternal, fetal, uterine, cervical, placental, hormonal and environmental etc. (Tabor and Alfirevic, 2010; Agarwal, 2012).

According to unani system of medicine abortion is a process in which the fetus is expelled out from the uterus before viability. The duration of pregnancy is usually 9 months and 12 days, but sometimes few days before or after delivery may occur, if deliver before 7 months the fetus does not survive or rarely may survive. Delivery in 6 months or in 8 months is harmful to the fetus. During abortion the women experience same events as at the time of delivery (Hakeem Ajmal Khan, Ibn Sina, Al Qanoon fil Tib, ynm).

Controversy: According to one group of physicians abortion usually occurs in early weeks because *Nutfa* (zygote) is formed from abnormal semen. Sometimes abortion occurs after 3 months of conception, but another group of physicians say that abortion usually occurs after 6 months only. It is due to diseases related to the mother or fetus. Cessation of sweating during pregnancy indicates the chances of abortion, especially when the fetus is developed.

Aetio-pathogenesis: The uterus performs its function with the capacities inherent in it. These capacities are varied and are specific. Apart from its nutritive capacity uterus is provided with certain distinct capacities that work for the development of fetus. Interference with fetal development, retention and expulsion related capacities results in defective development, defective retention or expulsion. The most important thing in this context is the temperamental balance between fetus and uterus temperament. Uterus retains only the fetus of its own favorable temperament. Any deviation from species temperament leads to activation of *Quwate Dafia* (Expulsive faculty) and causes abortion. Abnormal function of *Quwate Mussawira* (Morphogenetic faculty) is responsible for defective development and congenital abnormities. Over activity of expulsive capacity and hypoactivity of retentive power can also lead to expulsion of the fetus. The different causes that are described in classical unani text are related to these capacities and they interfere with one or more capacities and hence lead to defective functions. The ultimate goal is proper development and delivery of the baby. In later months fetal capacities can also play role in retention and expulsion of fetus from uterus. (Al Razi, 2011; Tabri, 2010; Abbas, 2005; Khan, 2011).

For successful conception normalcy of seminal fluids of both male and female is required. The male semen should be normal in its temperament and consistency. Any change in consistency or in temperament of semen interferes with conception and in formation of *nutfa* (zygote).

External pressure on uterus as seen in obesity or nutritional deprivation of fetus as occurs in lean and thin patients and after *istefragh* (Evacuation) may result

in abortion. Excessive cold and uterine fluidity interfere with fetal retention. Similarly hardness and irregularity of uterine surface may provoke expulsive power. Blood that nourishes the fetus may divert towards ruptured membranes and thus deprived the fetus of food. Fullness or congestion of utero-placental vessel with phlegm or flatus, fluid or blood may lead to vascular rupture and abortion. This phenomenon may cause easy placental detachment by increasing the weight of placenta. Similarly any obstruction in placental vessels deprives the fetal nutrition which results in abortion. On the basis of above pathological mechanism etiology can be described under following headings.

Etiology

Semen related

Raqiq or ghaleez mani (Fluidity or viscosity of semen)
Sue mizaj mani (Abnormal temperament)
(Ibn Sina, Al Qanoon fil Tibb, Akbar Arzani, 2002, Majoosi, 2010

Temperamental

Sue Mizaj Haar (Ill hot temperament of uterus)
Burudat Rehm (Coldness of uterus)
(Ibn Sina, Al Qanoon fil Tibb, Ajmalul Hasan Jurjani, 2010)

External or Psychological

Fear
Anxiety
Stress
Tension
Extreme happiness
Sudden mental trauma
(Abbas Mansurul Hasanul Qamri, 2005)

Environmental

Extreme cold and hot
(Akbar Arzani, 2002)

(A) Maternal causes

Elder or young age; Thin or morbid obese; General weakness; Malnutrition; Anemia; Indigestion; Addiction of alcohol; Acute diseases; Fever with chills; Worm infestation; Cholera; Severe diarrhea; Dysentery; Hysteria; Draining per vagina;

Hemorrhage; Sexually transmitted diseases like Syphilis and Gonorrhea; Trauma on abdomen or chest; Any painful conditions; Use of drugs in early pregnancy Like *Garam* (Hot), *Mazluq, Mushil* (Purgatives), *Mudir* (Diuretics), *Muqqai* (Emetic), *Mautis* (Sneezing produce), *Mmuqrijjaneen* (Abortificient), *Munavim* (Narcotics) etc.; Excess movements like jumping; Weight lifting; Strenuous execise; Excess cough; Procedures like *Fasd* (Blood letting), *Istifragh* (Excessive elimination of blood), Especiall yearly and late pregnancy; Any condition that produces *Laaza* (Irritation) of the uterus Like hot bathing, bathing for long time, prolong stay in bathroom and excessive intercourse (Hakeem Ajmal Khan, Haziq; Abbas Mansurul Hasanul Qamri, 2005; Al Razi, 2011).

B. Fetal causes : Fetal and Fetal Membranes

Intra uterine death; Intra uterine growth retardation; Multiple pregnancy; Big baby; Abnormal baby; Congenital anomalies of the fetus; Polyhydramnios; Weak placental attachment; Placental insufficiency; Rupture of membranes; Amino chorionic diseases; Defect in the membranes like lax or filled with fluidor tense membranes (Al Razi, 2011; Tabri, 2010; Abbas Mansurul Hasanul Qamri, 2005).

C. Uterine causes

Cervical incompetence; Syringing of *Mazluq* drugs in the uterus; *Sue Mizaj Rehm* (Ill temperament of uterus); *Riyah Rehm, Rutubat Rehm* (Excess of air or fluid in the uterus); *Warm Rehm* either soft or hard; *Sartan Rehm* (Carcinoma of uterus); *Qarahe Rehm* (Uterine ulcers); *Salabat Rehm* (Hardness of uterus); Hardness or irregular surface of the uterus; Defect in the uterus assmall uterus; Congestion of the uterus; *Suddain* the utero-placental vessels; *Rutubat mukhat* (Mucoid) *Balgham* (Phlegm) or *Riya* (Flatus) in the placental vessels; Ovarian tumors (Khan, 2011; Arzani, 2002; Majoosi, 2010).

Sign and Symptoms

General: Flushing of face, fever with chills, heaviness in the head and body, pain in eyes, abdominal pain, low backache, mild or heavy bleeding per vagina, loss of fetal movements and absence of fetal heart sounds.

Temperamental: In *Sue Mizaj Haar* the tense body, red and painful eyes, headache and burning sensation in the body.

Uterine: Irregular movements, burning and pain and irritation.

Breast related causes: Breast health depends upon fetal wellbeing and nutritional status of the body, milk secretion during pregnancy indicates weakness of the fetus.

Maternal: Bleeding per vagina, puffiness of face, excess of saliva, general weakness is indication of dominance of *Ratubat*.

Fetal: Irregular movements indicate fetal intra uterine growth retardation; loss of fetal movements indicates fetal death. Sometimes there is no cause of abortion except weakness and then symptoms of weakness are present (Hakeem Ajmal Khan, Haziq, Akbar Arzani, 2002,Majoosi, 2010,Akbar Arzani, Tibb Akbar).

Treatment

Principles of treatment

Bed rest is adviced in left lateral position; avoidance of jerky movements and vaginal as well as oral i.e. systemic use of astringent drugs are recommended. *Muqawi ghiza* (Nutritious diet) should be given. In case of flatulence use of *Mukhrij Balgham* (Phlegm elimination) and anti-flatulent drugs are advocated (Hakeem Ajmal Khan, HaziqIbn Sina, Al Qanoon fil Tibb Azam Khan, 2011, Akbar Arzani, Tibb Akbar).

In case of recurrent abortion

Before conception: Avoid external and psychological causes like fear, anxiety, worries etc. In case of internal causes induction of vomiting and use of *Munzij wa Mushil Balgham* Advia (drugs) for evacuation of excess body fluids have been in the practice. *Istafragh wa tahleel*- elimination of *rutubat* and dissolution of fluids should be done. *Muqawi badan* wa *rehm* measures should be adopted. Procedures like vomiting, purgatives are advised before conception only. So that the conception can occur easily and the fetus can retain properly (Hakeem Ajmal Khan, Haziq, Ibn Sina, Al Qanoon fil Tibb).

After conception: Fetal preservative measures are adopted such as avoidance of any external abortion inducing factor like extreme cold, heat, fear, stress, trauma etc. Relaxing measures should also be taken. After conception, use of *Hafiz Janeen drug* like *Majoon Hafiz Janeen* to prevent abortion, if abortion has already occurred then use of strong abortificients, emmenogogues and diuretics to expel the wastes from the uterus are advocated.

Inevitable abortion and after abortion

Advice abortificient and emmenogague drugs like *Abhal* (Juniperus communis Linn), *Sudab* (Ruta graveloens Linn.), *Halteet* (Ferula asafetida Linn.) etc. *Muqavi rehm advia* should also be given after the elimination of wastes is complete.

Advice and dietary recommendation

If the body constitution of the mother is weak then light and nutritious diets like half boiled egg and meat soups are recommended. They cause weight gain; in case of obesity for weight reduction less nutritious bulky diet is recommended.

After abortion the women should be more conscious because in comparison to normal delivery abortion is more painful condition and it may lead to many complications like heavy bleeding per vagina, fever, infection, uterine disease and even death. These maternal morbidities and mortalities can be prevented by appropriate treatment at appropriate time (Hakeem Ajmal Khan, Akbar Arzani, Tibb Akbar).

Conclusion

The literature of Unani medicine has wealth of treasure on abortion. Almost every author has described the abortion along with its necessary details. The description is so complete regarding etiology and principles of treatment that nothing could be added over last decades. From the writings of Unani physicians it is very much clear how much concern they were about mother and child health. Apart from this, they paid attention towards the well being of fetus in uterus. They believed that post delivery health of baby was some how linked with health and development in uterus. Hence detailed description regarding the measures for proper development and health of developing fetus is given. So many drugs are mentioned that preserve the fetus and protect it from various hazards. Knowledge about the susceptibility of fetus to various exogenous and endogenous hazards is well incorporated. Saying every drug should be avoided during first trimester. In this review scattered knowledge and description about the abortion and its consequences have been systematized in an easy form to comprehend organized way. The presentation is only in systematic way not explanatory. Only the classical books authored by well-known physicians were consulted leaving the commentaries and text book of later period.

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The Concept of *Nuzj* (Concoction) in Unani System of Medicine

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Abstract

The Unani Medicine is based on certain fundamental theories and concepts, such as Mizāj (temperament), Akhlāt (humours) and Quwā (Faculties) etc. *Nuzj* (concoction) is a concept of Unani Medicine, not simply a term. The process of *Nuzj* is related to *mādda* (matter) either normal or abnormal. Therefore, each type of *mādda* in the body passes through the process of *Nuzj* and thereafter the fate (assimilation or elimination) of mādda can occurs. Thus, the process of *Nuzj* plays an imperative role in the preservation and restoration of health. The material on this concept is scattered in classical Unani literature and not clarified. The present paper has attempted to focus the concept and to make it more elucidated.

Keywords: *Nuzj, Mādda,* Mizāj, Akhlāt, Quwā, Assimilation, Elimination.

Introduction

Nuzj (concoction) is a concept in Unani Medicine and the treatment of *muzmin māddī maraḍ* (chronic repletive diseases) depends on the concept of *Nuzj*. Literally, *Nuzj* means "*pakana*" (processing) (Arzani, 2010; Qamri, 2008) of *mādda-e-maraḍ* (causative matter). It prepares the *mādda-e-maraḍ* for accepting the effects of *ṭabī 'at* (Qamri, 2008) so that *ṭabī 'at* can get rid of *mādda-e-maraḍ* through the normal channels (Razi, 2000). Once *ṭabī 'at* becomes *ghalib* (dominant) on causative matter, it will surely maintain normalcy because *ṭabī 'at* in Unani Medicine is considered as *mudabbira badan* (supreme planner of the body) (Arzani, 2010; Jilani, 1998).

The process of *Nuzj* occurs slowly not suddenly in *mādda* either normal or abnormal (Arzani, 2010) and it requires *mu'tadil* (moderate) *ḥarārat-e-gharīziyya* (innate heat) (Ibn Rushd, 1987). The *mu'tadil ḥarārat-e-gharīziyya* is responsible for maintaining physiological functions of the body and its excess results in *iḥtirāq* (combustion) of *khilt* (humour) but when the innate heat is subnormal, it can not perform the normal metabolic process, therefore unable to give proper *Nuzj* in *mādda*.

Explanation of the concept of Nuzj

Nuzj is a normal process occurring in the body and it is the function of *ḥarārat-e-gharīziyya* (innate heat). It takes place according to the *tabakh* (digestion) of *ghidhā'* (diet) and occurs in stomach, liver and organs. This process needs the optimum innate heat of organs, if any deviation occurs in innate heat of organs due to accumulation of abnormal matter in organs, there is dire need of help of physician to use such drugs which can help in the process of *Nuzj* so that *ṭabī 'at* becomes *ghalib* (dominant) on *mādda* (Ibn Rushd, 1987) and can eliminate it with the help of *quwwat-e-dāfi 'a*

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(expulsive power) (Arzani, 2010). Therefore, the process of *Nuzj* helps in the elimination of causative materials out of the body easily and it plays an important role in the elimination of waste products which are responsible for the causation of disease (Qarshi, 2011).

The technical meaning of *Nuzj* of any *khilṭ* is the re-establishment of mu‘tadil *qiwām* (moderate viscosity) of *khilṭ*, so that it can easily be eliminated out of the body. Out of four humours of the body, *khūn* (blood) does not need *Nuzj* but other three humours *ṣafrā*' (bile), *sawdā*' (black bile) and *bhalgham* (phlegm) need *Nuzj* before evacuation. The *Nuzj* of *akhlāt-e-radīya* (abnormal humours) refers that after *Nuzj* they become capable of accepting the effects of *mushil* (purgative) drugs. The period of completion of *Nuzj* is different for different *khilṭ* (humour), such as *ṣafrā*' (bile) requires three days, *bhalgham* (phlegm) requires nine days and *sawdā*' (black bile) requires fifteen days. But, this period may vary if more than one *khilṭ* combine together. The process of completion of *Nuzj* does not depend only on *munḍij* (concoctive) drugs because God the great has given a hidden power to every individual known as *ṭabī ‘at* which is capable for maintaining normalcy, for giving *Nuzj* (processing) and for evacuating the waste materials out of the body (Arzani, 2010). Hence, the above discussion clearly indicates that the physicians by prescribing *munḍij* drugs only help *ṭabī ‘at*, so that the process of *Nuzj* can be completed easily otherwise *ṭabī ‘at* will complete the process without *munḍij* drugs, but it will take longer time.

The change in *nabḍ* (pulse) and *bawl* (urine) represents the stages of process of *Nuzj* either complete or incomplete (Qarshi, 2011). Therefore, physicians, by identifying changes in pulse and urine with their own skills, can determine the process of *Nuzj* and proceed for further treatment accordingly. There are some controversies among Unani scholars in relation to *istifrāgh-e-mādda* (elimination of waste materials) and administration of *munḍij* drugs for the completion of the process of *Nuzj* in acute and in chronic diseases. Jalinus (Galen) and most of Unani scholars are in favour of waiting the completion of process of *Nuzj* for *istifrāgh* (elimination) except, if the disease is very acute, *Nuzj* is not awaited and physician can proceeds for *istifrāgh*. Hippocrates also favours this view (Ibn Rushd, 1987). The examples of diseases which require *Nuzj* before *istifrāgh*, *are Fālij* (hemiplegia), *Laqwa* (facial palsy) and *Malankhūliya* (melancholia) etc.(Ghani, 2010). The disease, *Ḥummā Sūnūkhus* (synochus fever), does not need *Nuzj* before *istifrāgh* (Arzani, 2010) .The above explanation clearly indicates that the prescription based on *munḍij* (concoctive) and *mushil* (purgative) drugs completely depends upon the skills and experience of the physicians.

Nuzj kamil (perfect concoction) occurs in liver and *kamal-e-Nuzj* (extreme concoction) indicates the *zamān-e-intiha* (peak phase) of disease. The symptoms of *Nuzj* can be observed in *amrāḍ-e-ras* (diseases of head) by *ruṭūba*t (fluid) coming out from nose, in intestinal diseases by *barāz* (faeces) and in liver diseases by *bawl* (urine). In the

same way, other organs diseases can be assumed. By observing these changes, physicians can guess the stages of *Nuzj*, such as occurrence of changes in urine indicates initiation of the process of *Nuzj*. If these changes are towards *rasūb-e-maḥmūd* (desirable sediments) then *Nuzj* occurs otherwise *'ufūnat* (infection) will develop. The *rasūb-e-maḥmūd* (desirable sediments) can be identified by its colour, it is white and glimpse in colour and sediments are at the bottom of urine. The presence of *rasūb* (sediments) on the surface of urine indicates the initiation of *Nuzj maḥmūd* (desirable concoction) and its presence in middle of urine indicates the *ausat Nuzj* (average concoction). The *rasūb* opposite to white colour are considered *radī* (abnormal) (Qamri, 2008). Zakariya Razi says that the appearance of *rasūb* in urine is due to the process of Nuzj. He further delineates that in winter season more *rasūb* appear in urine because this season is favourable for excellent Nuzj (Nadvi, 1995). In this way, the physicians with their own skills can observe the process of *Nuzj* in *nabḍ* (pulse) also, because change in character of *nabḍ* indicates the process of *Nuzj* (Qarshi, 2011). Therefore, the changes in the character of *nabḍ* (pulse), *bawl* (urine), *barāz* (faeces) can be considered as parameters for the assessment of *Nuzj*. The perception of these changes in the character of the *nabḍ, bawl* and *barāz* by the skilled physicians comes through long experience.

Importance of Nuzj

Once the process of *Nuzj* is completed, the patients are free from the fear of death or bad prognosis and do not die with the same disease (Qamri, 2008). Because the *ṭabī 'at* becomes *ghalib* (dominant) on *mādda-e-maraḍ* (causative matter) and it will surely expel the causative matter out of the body and restores the health.

It can be observed that the physicians mostly advice bed rest. What is the aim of physician by advising bed rest? The Unani Medicine believes that rest is responsible for the absolute indulgence of *ṭabī 'at* towards the completion of process of *Nuzj-e-akhlāt* (concoction of humours). Ibn Sina in support of this says that *ṭabī 'at* at night gives excellent *Nuzj* in *mādda-e-maraḍ* (causative matter) (Ibn sina, 2010). Therefore, it is clear that by advising rest we are only supporting *ṭabī 'at* in its complete engagement in the process of *Nuzj*.

Conclusion

Nuzj is an important concept in Unani Medicine carried out by *ṭabī 'at* with the help of *ḥarārat-e-gharīziyya* (innate heat) in order to maintain the existing health and to restore it, if lost. After the process of *Nuzj*, *ṭabī 'at* sense the *mādda* (matter) and takes the action, either for assimilation or for elimination accordingly. Therefore, the process of *Nuzj* occurs in both types of *mādda* (matter) i.e. in normal and in waste materials. In diseased condition Unani physicians by prescribing *munḍij* (concoctive) drugs

merely help *ṭabī ʻat* to become dominant on *mādda-e-maraḍ* (causative matter) and eliminate it from the body, so that equilibrium can be achieved. Thus, the recommendation of *munḍij* and *mushil* drugs in the treatment of *māddī* disease, especially in chronic diseases plays a pivotal role in Unani Medicine. For Unani practioners, it is a matter of main concern while treating any disease.

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Therapeutic Evaluation of a Unani Formulation (*Safoofe Muhazzil Khaas*) in the Management of *Samane Mufrat* (Obesity)

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Abstract

Samane Mufrat (Obesity) is a worldwide problem and is the most common disorder of nutrition in affluent societies. It is a state of excess adipose tissue mass more than 20% of body weight, becoming leading risk factor for human health. *Samane Mufrat* is described in classical Unani literature in detail with its successful management. The Unani formulation *Safoofe Muhazzil Khaas* advocated in *Kitabul Mansoori* for the purpose is used for this clinical trial. The patients were advised dietary restrictions and brisk walk in both test group and placebo control group. Test drug formulation as well as placebo, both showed statistically significant improvement, with a little better improvement by test drug formulation. This formulation is clinically effective, but requires long term therapy to achieve appreciable improvement in obesity.

Keywords: Samane Mufrat, Obesity, Safoofe Muhazzil Khas

Introduction

Samane Mufrat (Obesity) is a worldwide problem and is the most common disorder of nutrition in affluent societies (Chugh, 2011). It is a state of excess adipose tissue mass (Longo *et al.*, 2012; Mohan, 2013; Papadakis and Mcphee, 2009; Gelder *et al.*, 2006) characterized by excessive accumulation of fat in the subcutaneous and deep tissue of the body, usually 20% or more of an individual's body weight (Mohan, 2013). *Samane Mufrat* is a complex, multifactorial chronic disorder involving environmental (social and cultural), genetic, physiologic, metabolic, behavioral, and psychological components.

The World Health Organization (WHO) describes global obesity, or "globesity" as one of the top 10 risks factors to human health. The health consequences of obesity range from a number of nonfatal complaints affecting the quality of life, such as respiratory difficulties, musculoskeletal disorders, infertility, and increased risk of high levels of disability, to complaints that lead to an increased risk of premature death including diabetes mellitus type 2, gallbladder disease, cardiovascular problems (hypertension, stroke, and congestive heart disease), and certain cancers (endometrial, breast, and colon). Almost 30-65% of adult urban Indians are reported to be overweight (BMI>=25) or obese (BMI>=30) or have central obesity (Mathur, 2011).

According to classical Unani literature *Samane mufrat* can be general and local as well and is caused by *murattab wa muragghan ghiza* (oily food) (Razi, 1999). In *Samane mufrat hararat-e-ghrizia* diminishes slowly and that is why, obese persons die early than others (Majoosi, 2010) and there is strong co-relation

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between excessive obesity and cardiovascular, cerebrovascular, respiratory and neurological complications (Qamari, 2008; Chandpuri, 1998; and Arzani, YNM). As far as the management is concerned, diet therapy is must along with medicines to reduce weight (Kantoori, 2010 and Jurjani, 2010). *Ibn-e-Sina* focuses on the *taqleel-e-ghiza* (dietary restriction) as the important tool for the purpose, and his treatment was an appetite suppressant made of almonds and beef suet, marsh mallow root, and oil of violets, taken for ten days to abate hunger (Kantoori, 2010) it is also advised that obese persons should avoid taking fatty diets, cooked in oil (Baghdadi, 2005).

Unani physicians recommend the drugs possessing *Muhazzil, Mulattif, Mushil, Mudir and Musakhin* properties in order to reduce weight for the management of *Samane Mufrat.* Single drugs for the purpose are *Luk, Marzanjosh, Tukhme Suddab, Karafs, Juntiyana, Lehsun, Zarawand, Nankhah, Kamoon, Badyan, Sandroos, Filfil siyah, filfil moya, Bisbasa, Aamla, Tukhme Khyarain* etc.

Murakkab Drugs for the purpose are *Safoofe Muhazzil, Safoofe Muhazzil Khaas, Habbe Tinkar, Majoone falafali, Jawarish kamooni, Jawarish bisbasa, Dawa-ul-luk, Anqardiya, Asanasiya, Sikanjabeen,Baladari, Amroosiya, Sanjarniya, Itrifal Sagheer* etc. The compound formulation *Safoofe Muhazzil Khaas* is one of them having most of the single drugs possessing anti-obesity property. Most of the ingredients of Safoofe Muhazzil Khaas are having the following medicinal properties making the formulation a reliable formulation: Mufatteh Sudad, Mulattif, Muqawwi-e-Hararat-Ghrizee, Musakhin, Muqawwi Meda, Mudir Baul, Mushtahi, Kasir Riyah, Qaat-e-akhlat-e-ghliza, Jaali, Muqawwi-e-meda wa Jigar, Mujaffif-e-ratubat-e-badan (Ansari, 1930; Azeez, 1948; Qamri, 2008; Chatterjee, 1995; Anonymous, 2008).

Material and Method

This study was single blind placebo controlled clinical trial conducted in department of Moalejat, Ajmal Khan Tibbiya College hospital during 2012-2014. A Unani compound formulation *Safoofe Muhazzil Khaas* (Table: 1) recommended by *Abu Bakar Mohammad bin Zakaria Razi* was selected as test drug to assess its efficacy in the management of *Samane Mufrat* (Razi, 1999). The test drug formulation was procured from the Dawakhana Tibbiya College, Muslim University, Aligarh. The original formulation is in powder form, but for the purpose of study this powder was transformed into tablets. The test drug was administered in the dose of 5 gm in the morning, whereas wheat flour tablets were given as placebo in the same dose for a period of 2 months. Patients in both groups were also advised exercise (brisk walk for 20-30 minutes according to the condition of the patient) and low calorie diet (1200 – 1800 K. cal /day).

Table 1: Safoofe Muhazzil Khas (Razi, 1991)

S.No.	Name of the drug	Botanical/ Scientific name	Ratio
1.	*Ajwain*	*Trachyspermum ammi* (Linn.) Sprague	1 part
2.	*Suddaab*	*Ruta graveolens* Linn.	1 part
3.	*Zeera*	*Carum carvi* Linn.	1 part
4.	*Marzanjosh*	*Origanum majorana* Linn.	1 part
5.	*Badiyan*	*Foeniculum vulgare* Mill.	1 part
6.	*Krafs*	*Apium graveolens* Mill.	1 part
7.	*Luk maghsool*	*Cocos lacca*	2 part
8.	*Bura Armani*	*Armenian bole*	1/4 part

Initially 48 patients were randomly allocated into test and placebo group. 5 patients in test group and 3 patients in placebo groups were dropped out leaving behind 25 patients in test and 15 patients in placebo group.

a) Inclusion criteria

Patients belonging to 15-60 yrs of age, BMI between 25- 35 kg/m^2, patient able to participate in the study and ready to follow the instructions and to sign the consent form, patients with symptoms complex that consists of any tow of the following clinical features were included in the study:

Restricted movement, joints pain, weakness and lethargy, dyspnoea, and palpitation

b) Exclusion criteria

Patient below the age of 15 and above the age of 60, pregnant and lactating women, patients with severe cardiovascular disease, severe renal disease, severe hepatic disease or any chronic disease, hypothyroidism, BMI > 35 kg/m^2, patients who refused to give the written informed consent for the study were not included in the study.

The selection of patients and the efficacy of the test drug were assessed on the basis of clinical examination and laboratory investigations used for the purpose.

Observations and Results

For the purpose of statistical analysis repeated measure ANOVA was used for intra-group comparison; whereas Kruskal Wallis test was used for inter group comparison as the two groups were of different sample size.

Body weight

The mean body weight in test group at 0 day was 73.8±1.1 (Kg), which reduced to 71.2±1.1 (Kg) at the end of trial, on statistical analysis the value of $p<0.001$, hence the result was significant. In placebo group mean body weight was reduced from 72.6±1.4 (Kg) to 70.5±1.3 (Kg), the result was found statistically significant ($p<0.001$) (Table 2)

For inter group comparison $p>0.05$, hence difference between two groups was not significant.

BMI

The mean BMI in test group at 0 day was 30.1±0.4 (kg/m^2), which reduced to 29.1±0.4 (kg/m^2) at the end of trial, on statistical analysis the value of $p<0.001$, hence the result was significant. In placebo group mean BMI was reduced from 29.6±0.4 to 28.7±0.3 (kg/m^2), the result was found statistically significant ($p<0.001$) (Table 2).

For inter group comparison $p>0.05$, hence difference between two groups was not significant.

Waist Circumference

The mean Waist circumference in test group at 0 day was 93.6±1.2 (cm), which reduced to 92.6±1.2 (cm) at the end of trial, on statistical analysis the value of $p<0.001$, hence the result was significant. In placebo group mean waist circumference was reduced from 93.4±1.4 (cm) to 92.6±1.5 (cm), the result was found statistically significant ($p<0.001$) (Table 2).

For inter group comparison $p>0.05$, hence difference between two groups was not significant.

Lipid Profile

Serum Cholesterol

The mean serum cholesterol in test group at 0 day was 202.2±8.5 (mg/dl), which reduced to 189.8±8.1 (mg/dl) at the end of trial, on statistical analysis the value of $p<0.001$, hence the result was significant. In placebo group mean serum cholesterol was reduced from 201.5±9.4 to 193.8±9.7 (mg/dl), the result was found statistically significant ($p<0.001$) (Table 3).

For inter group comparison $p>0.05$, hence difference between two groups was not significant.

Table 2: Effect of test drug vs. placebo on different parameters of obesity (Mean±SEM)

Group	Body weight (Kg)		Body Mass Index (Kg/m^2)		Waist Circumference (cm)	
	0 day	60th day	0 day	60th day	0 day	60th day
Test	73.8±1.1	71.2±1.1	30.1±0.4	29.1±0.4	93.6±1.2	92.6±1.2
n=25	P<0.001		P<0.001		P<0.001	
Placebo	72.6±1.4	70.5±1.3	29.6±0.4	28.7±0.3	93.4±1.4	92.6±1.5
n=15	P<0.001		P<0.001		P<0.001	

On applying Kurskal wallis test between test and placebo for body weight p>0.05, BMI p>0.05 and Waist circumference p>0.05

Table 3: Effect of test drug vs. placebo on Lipid Profile (Mean±SEM)

Group	Serum Cholesterol (mg/dl)		Serum Triglyceride (mg/dl)		HDL (mg/dl)	
	0 day	60th day	0 day	60th day	0 day	60th day
Test	202.2±8.5	189.8±8.1	189.5±12	171.5±12	37.2±1.3	45±1.4
n=25	P<0.001		P<0.001		P<0.001	
Placebo	201.5±9.4	193.8±9.7	155.2±8	144.1±9	39.4±1.5	45.4±1.5
n=15	P<0.001		P<0.001		P<0.001	

On applying Kurskal wallis test between test and placebo for Serum cholesterol p>0.05, Serum triglyceride p>0.05 and HDL p>0.05

Triglyceride

The mean serum cholesterol in test group at 0 day was 189.5±12 (mg/dl), which reduced to 171.5±12 (mg/dl) at the end of trial, on statistical analysis the value of p<0.001, hence the result was significant. In placebo group mean serum cholesterol was reduced from 155.2±8 to 144.1±9 (mg/dl), the result was found statistically significant (p<0.001) (Table 3).

For inter group comparison p>0.05, hence difference between two groups was not significant.

HDL

The mean HDL in test group at 0 day was 37.2±1.3 (mg/dl), which increased to 45±1.4 (mg/dl) at the end of trial, on statistical analysis the value of p<0.001,

hence the result was significant. In placebo group mean HDL was increased from 39.4±1.5 to 45.4±1.5 (mg/dl), the result was found statistically significant ($p<0.001$) (Table 3).

For inter group comparison $p>0.05$, hence difference between two groups was not significant.

Discussion and Conclusion

In this study the management of obesity is based on tripartite approach of treatment *(Ilaj bil Ghiza, Ilaj bit Tadbeer* and *Ilaj bil Dawa)*. Obesity is a chronic disorder and develops due to deposition of excessive fat and accumulation of morbid matter (*Ghalbae Akhlat-e-galeeza*). The dietary restriction and physical exertion is the mainstay in the management of obesity apart from the medicines. In one hand dietary restriction and physical exertion increases the consumption of body fat to provide energy and thereby helpful in reducing body weight and on other hand the Unani drugs used in the management help to evacuate the morbid matter particularly *Akhlat-e-ghaleeza* from the body.

Table 4: Safety Assessment comparison for both groups (Baseline vs. 60th day)

Parameters		Test group (n=25)		Placebo group (n=15)	
		0 day	60th day	0 day	60th day
		Mean±SEM	Mean±SEM	Mean±SEM	Mean±SEM
Hb%		11±0.2	11±0.2	11.78±0.3	11.7±0.3
TLC		8164±390	8028±400	7073±503	7300±450
DLC	P	50±1.8	45±2	39±2	39±2
	L	37±1.5	30±1.6	35±1.5	35±1.5
	E	3±0.2	2.5±0.3	2.8±0.4	2.8±0.4
	M	2.1±0.1	2.6±0.2	1.4±0.4	1.4±0.4
	B	0	0	0.1±0.9	0.1±0.9
ESR		30±2	28±1.9	27±3	26±2
S. Bilirubin		0.7±0.05	0.5±0.04	0.7±0.04	0.7±0.05
AST		32±2	28±2	31±2	29±2
ALT		32±1.5	31±1.5	32±1	48±2
Alk. Phos.		128±3	126±2.3	134±3	125±3
S. Creatinine		0.83±0.03	0.7±0.04	0.8±0.03	0.6±0.03
Blood Urea		28±1.4	28±1.5	32.1±1	28±2
B. Sugar (R)		116.25±3	108.68±3	128±4	123±4

Repeated measure ANOVA test is applied between 0 day and 60th day in both groups and for both groups the value of $p>0.05$, hence not significant

When data obtained were statistically analyzed for assessment of the efficacy of test drug formulation and placebo by using repeated measure ANOVA test for intra-group comparison it was found that the result was significant for all the parameters in both groups. On inter-group comparison by Kruskal Wallis test it was found statistically insignificant (p>0.05); meaning therefore the test drug formulation and placebo both have almost equal efficacy on reducing obesity, however the test drug formulation exhibited a little better result.

Significant effect on reducing body weight in placebo group may be due to strict dietary restriction and moderate exercise which was advised in both groups. Further, in test group a little better improvement may be attributed to the *Muhazzil* effect of *Luk maghsool* (Kantoori, 2010; Baitar, 2003; Hakeem, 2002; Naseer, YNM; Ghani, YNM), *Mulattif* action of *Marzanjosh* (Baghdadi, 2005; Ghani, YNM), *Hazim wa Mulattif* effect of *Ajwain, Zeera* and *Badiyan* (Ghani, YNM; Baitar, 2003), *Ajwain, Zeera, Karafs* and *Marzanjosh* also have *Musakhin* effect thus increase BMR, and *Qate akhlate ghaliza* properties of *Bora Armani* (Kantoori, 2010; Baitar, 2003; Hakeem, 2002; Kabeeruddin, YNM and Naseer, YNM). The above mentioned diverse pharmacological action of drug component of the formulation might complement or synergise each other and facilitate the anti obesity effect.

The significant improvement in BMI and Waist circumference is directly associated with reduction of body weight. The reduction in body weight in obese persons is mainly due to burning of the accumulated fat. Therefore, the reduction in waist circumference may be attributed to the dissolution of body fat and more precisely abdominal fat due to above mentioned medicinal properties of the Unani formulation and strict diet restriction with physical exertion as advised.

Lipid Profile

There were statistically significant reduction in the levels of Serum cholesterol and Serum triglyceride in both test group as well as in placebo group (p<0.001). For inter group comparison it was found insignificant (p>0.05). However, a little better result was seen in test group, this result may be due to lipid lowering effect of the main ingredient of test formulation *Luk maghsool* which possess *muhazzil* (weight reducing) property that is probably achieved by increased utilization of fat for energy production (Kantoori, 2010; Baitar, 2003 and Ghani, YNM), moreover increased physical exertion is associated with increased energy consumption that might be provided by burning of body fat.

According to the concept of Unani system of medicine fat is concerned as *Akhlate-e-ghaleeza,* and one of the drug components *Bora armani* is *Qate akhlat-e-ghaleeza*, hence this medicinal effect might reduced the serum cholesterol and

triglyceride (Kantoori, 2010; Baitar, 2003; Hakeem, 2002; Kabeeruddin, YNM and Naseer, YNM).

As far as the HDL is concerned, it is a good lipid and its quantity was increased in appreciable quantity, this achievement may be attributed to the physical exertion in the form of brisk walk as advised in both groups.

Body weight, BMI and WC are standard objective parameters for the assessment of efficacy of test drug and placebo in the patients of obesity. The overall improvement in obesity may be due to reduction in Body weight, BMI and WC as discussed above.

Several studies support our contention that dietary modification reduces energy intake and increased physical activity requires greater energy, ultimately required energy is provided by burning of accumulated fat and thereby reduces body weight, on other hand the Unani drugs used for the purpose evacuate the morbid matter particularly *Akhlate ghaliza* from the body. Though all the subjective and objective parameters along with laboratory findings did not come to the normal limits but the improvement is towards normalcy as the duration of therapeutics is shorter (2 months). If the same treatment be continued for a longer period, say 6 months, the results might be better, appreciable and in the normal range.

In this study safety parameters (Haemogram, TLC, DLC, ESR, LFT, and RFT etc.) were also taken into account to rule out any adverse effect (Table: 4) and after statistical analysis the drug formulation was declared as safe. During entire period of the study, no adverse effect was reported by patients.

In the light of above discussion it can be concluded that test drug formulation *Safoofe Muhazzil Khaas* produced a little better anti obesity effect without any side/ adverse effect as compared with the placebo, and it is further proposed to study the same formulation for a longer period to get better results to declare it as anti-obesity formulation.

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Comparative Study of Unani Formulations in the Management of *Zeequn Nafas Sho'bi* (Bronchial Asthma)

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Abstract

Although much progress has been made in our understanding of Bronchial Asthma over the past decades, asthma remains frequently encountered condition challenging to physicians. The burden of this disease to the government's healthcare systems, families and patients is increasing worldwide. The etiology of asthma is complex and multi factorial which involves the interaction between genetic factors and environmental stimuli.

Three Studies with Unani drug combinations were taken to compare their efficacy in the management of this disorder. Though all the drug combinations were proven to be effective individually but their comparative study showed the better choice to improve the clinical parameters of bronchial asthma was the Gul-e-Zoofa, Berg-e-Aroosa & Aslus-Soos.

Keywords: Unani drug, Asthma, Gul-e-Zoofa, Berg-e-Aroosa, Aslus-Soos

Introduction

Zeequn Nafas Sho'bi (Bronchial Asthma) is a dangerous lung's disease, which was known to mankind since the time of Hippocrates. Hippocrates was the first to name this disease as 'panting' which means breathlessness (Wise *et al.*, 1985). Later on many Unani scholars keenly studied about Asthma and mentioned it in their books.

Although much progress has been made in our understanding of bronchial asthma over the past decades, but it remains frequently encountered condition challenging to physicians (Anonymous, 1997), the burden of this disease to the government's healthcare systems, families and patients is increasing worldwide (Anonymous, 2005). The etiology of bronchial asthma is complex and multi factorial which involves the interaction between genetic factors and environmental stimuli (Maddox, Schwartz, 2002).

Etio-pathologically three important changes have been identified in bronchial asthma viz. constriction of the bronchial muscle, excess mucus secretion and mucosal oedema in response to hyper-responsiveness. Therefore, its line of Treatment also based on the followings:

1. Bronchodilators to relieve bronchospasm
2. Anti-hypersensitivity drug, to stop hyper-responsiveness
3. Expectorant to eliminate excess mucus

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The Unani scholars have successfully treated this disease by herbo- minerals and even today the present scholars are following them. The western medicine has invented several bronchodilators and corticosteroids thus made the treatment much easier but apart from all these advancements, the complete treatment of bronchial asthma is yet to be achieved.

In Unani system of medicine, management of *Zeequn Nafas Sho'bi* is fully described on the basis of its etiopathogenesis including its symptoms and signs. The principle of its management is based on humours involved, etiology of the disease and its severity. According to Unani concept, the main cause of this disease is accumulation of *Balgham-e-Lazij* (stichy phlegm) in airways causes cough and breathlessness. *Nuzj* (Concoction) and *Tanqia* (evacuation) of *Balgham-e-Lazij* (stichy phlegm) is the basis for the management of ailment.

To facilitate easy elimination of abnormal humour, it is necessary to change the viscosity of ailing humour to its physiological state is called *Nuzj* (concoction). To achieve the above purpose selective *Munzijat-e-Balgham* (phlegm concotives) and *Mushil* (purgatives) are being used. Sometimes *Muqiat* (emetics), *Mulattifat* (demulscents) and *Munaffis-e-Balgham* (expectorants) are also used (Avicena, 1992; Razi, 1998; Jurjani, 1878; Kabiruddin, 2000; Khan, YNM). As precautionary measures phlegmagogue and allergent diets/drugs should be avoided (Barkatullah, 1997). Light diet should be prescribed to the patient and they should be kept away from humid places (Razi, 1998).

Apart from systemic pharmacotherapy, Hakeem Akbar Arzani was the first who used the medicine in the form of *Bakhoor* (smoke) which is very much similar to drug inhalation in recent times (Arzani, YNM).

Purpose of the Study

Objective of this study was to critically review and analyze the three Unani drug formulations investigated earlier and put forth the most promising drug combination that can be reassessed for its efficacy by using more comprehensive and standard protocols.

Material and Method

This retrospective study was carried out to elaborate the promising drug combination studied in the past for the treatment of *Zeequn Nafs Shoabi* (Bronchial Asthma). The study was divided into three groups, namely, I, II and III. In Group I the test drug was decoction of Gul-e-Zoofa (*Hyssopus officinalis Linn.*), Berg-e-Aroosa (*Adhatoda vasica* Nees) and Aslus-Soos (*Glycyrhiza glabra* Linn.), in Group II the test drug was decoction of Irsa (*Iris ensata* Thunb.), Mulethi

(*Glycyrhiza glabra* Linn.), Kakraseenghi (*Pistacia integerrima* Stewart ex Brandis) and Zanjabeel (*Zingiber officinale* Rosc.) and in Group III the test drug was a powdered combination of Qaranfal (*Eugenia caryophylla* Thunb.), Irasa (*Iris ensata* Thunb.), Zanjabeel (*Zingiber officinale* Rosc.) and Maghz-e-Amaltas (*Cassia fistula* Linn.). All the drugs were given orally twice a day for a period of forty two days in Group I and II while sixty days in Group III.

Observations and Results

Effect of test drug combinations on Chest Expansion

In the study the mean expansion of chest recorded before treatment was 1.2 ± 0.4 cm, 1.61 ± .32 cm and 1.3 ± 0.2 cm in group I, II and III respectively. After treatment it was increased to 1.4± 0.5 cm, 2.21± .41 cm and 1.5± 0.5 cm in the same sequence. On applying paired't' test, the effect of our drugs in increasing the expansion of chest was highly significant ($p < .001$) (Table 3).

Group II had shown maximum improvement followed by Group I while Group III had shown least improvement in chest expansion.

Table 1: Test drug combinations used

Drug Combination	Form of Test Drug Combination	Ingredients	Botanical Identity
I	Decoction	Gul-e-Zoofa	*Hyssopus officinalis* Linn.
		Berg-e-Aroosa	*Adhatoda vasica* Nees
		Aslus-Soos	*Glycyrhiza glabra* Linn.
II	Decoction	Irsa	*Iris ensata* Thunb.
		Aslus-Soos	*Glycyrhiza glabra* Linn.
		Kakraseenghi	*Pistacia integerrima* Stewart ex Brandis
		Zanjabeel	*Zingiber officinale* Rosc.
III	Powder	Qaranfal	*Eugenia caryophylla* Thunb.
		Irsa	*Iris ensata* Thunb.
		Zanjabeel	*Zingiber officinale* Rosc.
		Maghz-e-Amaltas	*Cassia fistula* Linn.

(Shoaib, 2003; Khan, 2006; Naseer, 2008)

Effect of test drug combinations on Respiratory Rate

The mean respiratory rate recorded before the use of the test drug combinations was 24.6 ± 3.1 per/min., 24.8 ±2 .1 per/min. and 23.1 ± 1.1 per/min in Group I, II and III and it was reduced to 19.0± 0.9 per/min., 20.1± 1.5 per/min. and 20.0± 0.5 per/min respectively at the end of study. The effect of our drugs in reducing respiratory rate was significant (p <.001) (Table 3).

Among them Group I had shown maximum improvement followed by Group II while Group III had shown least improvement in respiratory rate.

Effect of test drug combinations on Breathlessness

The mean number of attacks of breathlessness per week recorded before the use of test drug combinations was 5.7± 2.6, 5.0± .83 and 5.8± 1.8 and it was reduced to 0.5± 0.7, 0.56± .67 and 0.6± 0.4 in Group I, II and III respectively at the end of study. The effect of our drugs in reducing the number of attacks per week was significant (p <.001) (Table 3).

Similar to above, it was observed that Group I had the maximum mean improvement in respiratory rate followed by Group II and III.

Effect of test drug combinations on Peak Expiratory Flow Rate (PEFR)

The mean peak expiratory flow rate recorded at the time of commencement of study of test drug combinations was 205 ± 73.7 lit. /min and 271 ± 60.0 lit. / min in Group I and II. At the end of study, it was increased to 378± 70.3 lit. /min. and

Table 2: Regimen of test drug combinations used

Drug Combination	Ingredients of Test Drug Combination	Form of Drug Combination	Dosage and Timings	Duration
I	Gul-e-Zoofa, Berg-e-Aroosa & Aslus-Soos	Decoction	4 grams each drug 12 Hourly	42 days
II	Irsa, Mulethi, Kakraseenghi, Zanjabeel	Decoction	6 grams each drug 12 Hourly	42 days
III	Qaranfal, Irasa, Zanjabeel, Maghz-e-Amaltas	Powder	3, 6, 3 & 4 grams each drug 12 Hourly	60 days

(Shoaib, 2003; Khan, 2006; Naseer, 2008)

354± 61.4 lit./min. in the same sequence. The PRFR parameter was not included in the group III. The statistical analysis shows that the effect of our drugs in increasing PEFR was significant ($p < .001$) (Table 3).

Maximum mean improvement in PEFR was noted in Group I followed by Group II.

Effect of test drug combinations on Eosinophil Count

The mean eosinophil count recorded before the start of the study was 7.0 ± 1.5 per cubic mm, 9.30 ± 2.6 per cubic mm and 13.63 ± 2.60 per cubic mm in Group I, II and III respectively. It was decreased to 4.6± 1.1 per cubic mm, 6.00± 1.89 per cubic mm and 12.94 ± 2.24 per cubic mm respectively at the end of study. The statistical analysis shows that the effect of our drugs in reducing eosinophil count was significant ($p < .001$) (Table 3).

Table 3: Effect of drug combinations on different parameters

S. No.	Parameter	Drug Combination I		Drug Combination II		Drug Combination III	
		Before Tt	After Tt	Before Tt	After Tt	Before Tt	After Tt
1.	Mean of Chest Expansion ± S.D. (cm)	1.2±0.4	1.4±0.5 t = -5.2; p < 0.001	1.61±.32	2.21±.41 t = -14.90; p < 0.001	1.3±0.2	1.5±0.5 t = -7.2; p < 0.001
2.	Mean Resp. Rate ± S.D. (Per min.)	24.6±3.1	19.0±0.9 t = 6.6; p < 0.001	24.8±2 .1	20.1±1.5 t = 14.2; p < 0.001	23.1±1.1	20.0±0.5 t = 7.3; p < 0.001
3.	Mean No.of attacks of Breathlessness ± S.D. (Per Week)	5.7±2.6	0.5±0.7 t = 10.9; p < 0.001	5.0±.83	0.56±.67 t = 8.4; p < 0.001	5.8±1.8	0.6±0.4 t = 11.2; p < 0.001
4.	Mean PEFR ± S.D. (Lit. /Min.)	205±73.7	378±70.3 t = 15.85; p < 0.001	271±60.0	354±61.4 t = 15.85; p < 0.001	-	-
5.	Mean Eosinophil Count ± S.D. (Cubic mm)	7.0±1.5	4.6±1.1 t = 9.7; p < 0.001	9.30±2.6	6.00±1.89 t = 11.95; p < 0.001	13.63± 2.60	12.94± 2.24 t = 2.33; p < 0.05
6.	Mean AEC ± S.D. (Cubic mm)	-	-	728.23± 166.13	509.30± 120.90 t = 11.48; p < 0.001	917.67± 221.84	877.80± 218.12 t = 2.32; p < 0.05

(Shoaib, 2003; Khan, 2006; Naseer, 2008)

During the study, Group II had shown maximum improvement followed by Group I while Group III had shown least improvement in eosinophil count.

Effect of test drug combinations on Absolute Eosinophil Count (AEC)

The mean absolute eosinophil count recorded before the start of treatment was 728.23 ± 166.13 per cubic mm and 917.67 ± 221.84per cubic mm in Group II and III respectively. It was decreased to 509.30± 120.90 per cubic mm and 877.80 ± 218.12 per cubic mm in the same sequence at the end of study. The AEC parameter was not included in the group I. The statistical analysis shows that the effect of our drugs in reducing absolute eosinophil count was significant ($p < .001$) (Table 3).

Maximum mean improvement was noted in Group II followed by Group III in reducing absolute eosinophil count.

Discussion

The test drugs used for the management of *Zeequn Nafas Sho'bi* (Bronchial Asthma) mainly have anti allergic, anti inflammatory, bronchodilatory and mucolytic effects. The studies conducted have different combinations having the drugs which possessed the above mentioned effects. Therefore all the test drug combinations have proved effective in the management of the disease.

The most effective drug combination is pertaining to Group I (Table-3). This combination showed maximum effect to increase mean peak expiratory flow rate (PEFR). Measurement of PEFR is the most important parameter along with forced expiratory volume in one second (FEV_1). PEFR could be considered authentic in absence of FEV_1. This effect can be attributed to presence of Aroosa (*Adhatoda vasica* Nees) and Aslus-soos (*Glycyrhiza glabra* Linn.) in the drug combination, which have bronchodilating and anti histaminic activities (Chopra *et al.*, 1980; Rastogi and Mehrotra, 1992; 1993; 1994). The role of Zoofa (*Hyssopus officinalis Linn.*) was also significant because, it has potent bronchodilatory and expectorant activity and helped in elimination of thick mucus. The improvement was also recorded in other parameters like respiratory rate, attacks of breathlessness, expansion of chest, eosinophil and absolute eosinophil count. The mechanism of action of the combination can be attributed to the effects of the drugs present in it like anti histaminic and bronchodilator of Aroosa (*Adhatoda vasica* Nees) and Aslus-soos (*Glycyrhiza glabra* Linn.) and expectorant property of Zoofa (*Hyssopus officinalis Linn.*) (Rastogi and Mehrotra, 1993; 1994).

Second most effective drug combination belongs to Group II (Table-3).This combination was most effective on expansion of chest. This overall effect may

be attributed to anti inflammatory and bronchodilating properties of Zanjabeel (*Zingiber officinale* Rosc.) and Aslus-soos (*Glycyrhiza glabra* Linn.) (Chaterjee and Pakrashi, 1995; Trivedi, 2004; Sharma, 2003). The anti allergic and mucolytic properties of the drugs present in drug combination are also responsible for the improvement in PEFR, breathlessness, respiratory rate and eosinophil count.

Third effective drug combination belongs to Group III. The expectorant property of Zanjabeel (*Zingiber officinale* Rosc.), Irasa (*Iris ensata* Thunb.) and Qaranfal (*Eugenia caryophylla* Thunb.) (Arzani, 1893; Kabiruddin, 1425-1433 H) and anti allergic property of Zanjabeel (*Zingiber officinale* Rosc.) and Irasa (*Iris ensata* Thunb.) (Khan, YNM; Khan, 1995) may be attributed to the improvement of different clinical parameters of bronchial asthma (Table 3).

Conclusion

To manage *Zeequn Nafas Sho'bi* (Bronchial Asthma), several Unani *Mufrid* (Single) and *Murakkab* (Compound) drugs are described in Classical Unani Literature and many studies have been carried out in different institutions to validate their efficacy on scientific parameters. On comparing some of these studies, it is being concluded that:

To manage bronchial asthma, all drug combinations were more or less effective but drug combination of Group I was found more effective in increasing the peak expiratory flow rate (PEFR) which is one of the important parameters to monitor the effects of drugs. The drug combination of Group II was found effective in the expansion of chest and drug combination of Group III also showed the significant effects on different clinical parameters.

Among the three drug combinations i.e. Gul-e-Zoofa, Berg-e-Aroosa, Aslus-Soos (Group I), Irasa, Aslus-Soos, Kakraseenghi, Zanjabeel (Group II) and Qaranfal, Irasa, Zanjabeel, Maghz-e-Amaltas (Group III), the best three was found to be Gul-e-Zoofa, Berg-e-Aroosa, Aslus-Soos (Group A) in the management of *Zeequn Nafas Sho'bi* (Bronchial Asthma).

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Pharmaco-Botanical Studies on Commercial Samples of Herbal Drugs for Their Identification*

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Abstract

Herbal drugs are procured by the pharmaceutical industries and other allied manufacturers from the commercial sources. In India, herbal drug trade is well established to fetch the demand of stockholders. Herbal drugs are resourced from various channels for marketing. The drugs are subject of intentional and unintentional adulteration or substitution. To address this problem, identification exercise of herbal material at various level is essential. Genuine herbal ingredientsare the basis of quality, safe and efficacious medicines.Pharmaco-botanical studies are the tools for identification of herbal drugs. In present studies, commercial samples were collected from vendors and subjected to identification employing pharmaco-botanical tools and matching their diagnostic macro and microscopic features with established quality standards.

Keywords: Pharmaco-botanical studies, Herbal drugs in commerce, Identification.

Introduction

The trade of herbal medicine and crude drugs are fast growing sectors in India. Large scale commercial production of herbal medicines in more than 9044 pharmaceuticals industries of Ayurvedic, Siddha, Unani and Homoeopathic system of medicines, increased the demand of herbal drugs (Anonymous, 2015). There are number of trading centres of herbal drugs, located in different parts ofIndia (Table-1). These trading hubs fetch the demand of herbal drugs to industry. The herbal drugs reach to trading centres through various channels after collection from forests, wild sources or cultivation (Tiwari *et al.*, 2014a, b; Ved and Goraya, 2008). The unprecedented demand and gap between supply and demands leads to adulteration and substitution in herbal drugs. The incidences of adulteration impact on qualityof herbal drugs and also on therapeutics of finished medicinal products. To address the problem of adulteration, quality standards of herbal drugs are indispensable tool. The quality standards are available as pharmacopoeial (regulatory) and non-pharmacopoeial standards. Pharmacopoeial standards are compiled in the Pharmacopoeia as individual monographs comprising descriptive and analytical bench marks to ensure the quality of herbal drug in respect of its identity, purity and strength. In India,Ayurvedic, Siddha, Unani, Homoeopathic and Indian Pharmacopoeia are published under the purview of Drugs Cosmetics Act 1940 and Rules thereunder. All these Pharmacopoeias have monographs on herbal drugs (Anonymous, 1940;

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Table 1: Important Herbal Drug Trade Centres in India

State	Trade Centres
Assam	Guwahati, Nagaon
Bihar	Rexaul, Patna
Chhattisgarh	Bilaspur, Raipur, Raighar, Dhamtari, Kanker, Jagdalpur, Dantewada
Delhi	Delhi
Gujrat	Sidhpur, Unjha, Ahmedabad, Godhra
Himachal Pradesh	Kullu, Mandi
Jammu & Kashmir	Srinagar, Jammu
Jharkhand	Sahibganj
Karnataka	Tumkur, Bangalore, Mysore
Kerala	Palakkad, Thrissur, Chochin
Madhya Pradesh	Gwalior, Sheopur, Shivpuri, Katni, Neemuch, Mandsaur, Sagar, Vidhisha, Bhopal, Jabalpur, Indore, Betul
Maharashtra	Mumbai, Navi Mumbai, Ratangiri, Kolhapur, Junnar, Pune
Manipur	Imphal
Meghalaya	Shillong
Orissa	Jharsuguda, Cuttack, Berhampur
Punjab	Amritsar, Ludhiana
Rajasthan	Jaipur, Jodhpur, Ajmer, Barmer, Sojat
Sikkim	Gangtok
Tamil Nadu	Krishnagiri, Chennai, Salem, Erode, Dindigul, Pudukkottai, Madurai,Virudhunagar, Tuticorin, Nagercoil
Uttarakhand	Dehradun, Haridwar, Ramnagar, Tanakpur
Uttar Pradesh	Moradabad, Bareilly, Hathras, Lucknow, Kanpur, Varanasi, Allahabad, Lalitpur
West Bangal	Kolkata, Siliguri

1971-2006; 1986-2008; 1998-2009; 2008-2011; 2009a, b; 2011a; 2014). Indian Council of Medical Research (ICMR) and Indian DrugsManufacturers Association (IDMA) have also published monographs on quality standards of herbal drugs but these are non-regulatory (Anonymous, 2003-2015; 2011b). Besides these

monographs, research papers on quality aspect of herbal drugs can be consulted for the identification of herbal drugs. In the present communication, commercial samples of herbal drugs were collected from different vendors and drug exhibitors of south India so as to ascertain their botanical identity sold in their region.

Material and Methods

The herbal drugsamples were collected from different vendors/exhibitors during Arogya fair (November, 2014) at Pragati Maidan, New Delhi and subjected to pharmaco-botanical studies (organoleptic, macro and microscopic) (Figure-A & B). These samples were soaked in water for 8-10 hours depending upon their



Figure A (1-6): Display of commercial herbal drugs by exhibitors

hardness to soften the tissues for hand sections. The observed macro and microscopic characters were matched with the diagnostic characters descripted in pharmacopoeias and other standard literature in order to confirm their botanical identity. The samples were also matched with the preserved drug specimens in Museum of PLIM, Ghaziabad.

Observations

The identification of collected commercial samples of herbal drugs are given in Table 2.

Table 2: Botanical Identification of Commercial Samples of Herbal Drugs

S. No.	Trade Name in South India	Other Trade Names	Botanical species	Morpho-logical Part	Reference
1.	Adalodakam (M)	Vasa, Arusa, Vasaka, Arusa (Bansa)	*Adhatoda vasica* Nees.	Stem	Prasad and Prabhu,1950
2.	Amukkuram (M)	Ashwagandha, Amukhra, Asgandha, Asgand	*Withania somnifera* (L.) Dunal.	Root	SPI-I
3.	*Aratha*(M)	Granthimula	*Alpinia calcarata* Rosc.	Rhizome	API- VI
4.	Banafshapatti (M)	Banafsa, Banaksha, Wild violet, Tryman, Vanpsa, Sweet violet, Nilapuspa	*Viola odorata* L.	Whole plant	HPI-IV
5.	Chakkarakolli (M)	Gudmar, Gudhmar, Gudmar, Bedki, Medhshingi	*Gymnema sylvestre* (Retz.) R.Br. ex Sm.	Whole plant	API -V
6.	Cheenapavu (M)	Parangipattai, Madhusnuhi, Chobchini, Parankiccakkai	*Smilax china* L.	Tuber root	SPI-I
7.	*Chukku* (M)	Sunthi, Ardraka, Zanjabeel	*Zingiber officinale* Roscoe	Dried Rhizome	SPI-I
8.	Elam (M)	Elathari, Elaichi, Cardamom, Malabar cardamom, Elakkai	*Elettaria cardamomum* Maton	Fruit	SPI-II

S. No.	Trade Name in South India	Other Trade Names	Botanical species	Morpho-logical Part	Reference
9.	Elavargam (M)	Twak, Dalchini, Gudutwaka, Tamalaka, Valkala, Kankutla, Lavangachakke, Lavangapatte	*Cinnamomum zeylanicum* Blume	Stem bark	API- I
10.	*Erattima-dhuram* (M)	Glycyrrhiza, Yasti, Asl-us-Soos, Athimathuram	*Glycyrrhiza glabra* L.	Stolon & Root	SPI-I
11.	Ezhilampaala (M)	Chattiyan, Kaadusaale, Saptaparni, Captaparanam, Kashim, Saptaparna Kashim (Chatim)	*Alstonia scholaris* (L.) R. Br.	Stem bark	API- I
12.	Gramboo (M) Karampoo	Lavanga, Launga	*Syzygium aromaticum* (L.) Merr. & L.M.Perry	Flower bud	SPI-I
13.	Jatamanji (M)	Jatamansi, Mansi, Bal char, Balchar, Laljari	*Nardostachys jatamansi* (D.Don) DC.	Rhizome	API- I
14.	Jeerakam (M)	Zeera,Zeera Safaid, Ajjikka, Dipaka, Dirghaka, Jeera, Jeeraka, Cumin	*Cuminum cyminum* L.	Fruit	SPI-I
15.	Kacholam (M)	Chandramoola, Kachoram, Gandhamoolaka	*Kaempferia galanga* Linn	Rhizome	QSIMP-7 (Anonymous, 2003-2015)
16.	*Kadukka*(M)	Halela Zard, Katukai, Haritaki Halela, Katukkai	*Terminalia chebula* Retz.	Fruit	SPI-I
17.	Kanhiram(M)	Kuchla	*Strychnosnux-vomica* L.	Seed	SPI-II
18.	Kanikonna (M)	Bahava, Bhavshenga, Amaltas, aargavadh, Khiyarshamber	*Cassia fistula* L.	Stem bark	SPI-I
19.	Karimjeera-kam (M)	Caraway, Kalazera, Seemaisompu	*Carum carvi* L.	Fruit	API- I

S. No.	Trade Name in South India	Other Trade Names	Botanical species	Morpho-logical Part	Reference
20.	Karivelam/ Karivelakam (M)	Talisa	*Abies webbiana* (Wall. ex D.Don) Lindl.	Leaf and stem	API- IV
21.	Karuvelam (M)	Babulla, Babul, Gum, Kikar, Barbura	*Acacia arabica* (Lam.) Willd.	Gum	IP,2007
22.	Kattuvizhalari (M)	Bhungi,Gaiya,Vaiva rang, Ambati, Nununiya	*Embelia robusta* Burm.f., Syn *Embelia tsjeriam-cottam* (Roem. & Schult.) A.DC	Fruit	API- I
23.	Katukurokini (M)	Kutki, Kadwi, Kedar Kadwi	*Pichrohiza kurroa* Royle ex. Benth.	Rhizome	SPI-I
24.	Kiriyatha (M)	Kalmegh, Kiryat	*Andrographis paniculata* (Burm.f.) Nees.	Stem	HPI-I
25.	Kothambala-yari (M)	Dhaniya	*Coriandrum sativum* L.	Fruit	SPI-I
26.	Kottam (M)	Kustha	*Saussurea costus* (Falc.) Lipsch.	Root	SPI-I
27.	Kunthirikkam (M)	Kundur, Pazhingu Sambhirani, Sallaki	*Boswellia serrata* Roxb. ex Colebr.	Gum (Exudate)	API- IV
28.	Kurumulaku (M)	Kali mirch, Maricha, Golmarich, Milagu, Murem	*Piper nigrum* L.	Fruit	SPI-I
29.	Kurunthotti (M)	Mahabala	*Sida rhombifolia L.*	Root	API- III
30.	Kutakappala/ Kutakapala-yari (M)	Kudassl, Kudashenga	*Holarrhena antidysenterica* (Roth) Wall. ex A.DC.	Seed	API- III
31.	Mahaniki-zhangu (M)	Sveta Sariva	*Decalepis hamiltonii* Wight & Arn.	Root	SPI-I
32.	Manhal (M)	Haldi, Turmeric, Haridra, Harita, Haali, Arashing, Haladi	*Curcuma longa* L.	Rhizome	SPI-I

S. No.	Trade Name in South India	Other Trade Names	Botanical species	Morpho-logical Part	Reference
33.	Maramanjal (M)	Daru-haridra, Mara manjal	*Coscinium fenestratum* (Gaertn.) Coleb.	Stem	API- V
34.	Masikka (M)	Manzuphal	*Quercus infectoria* G. Olivier	Gall	SPI-II
35.	Mathalathodu/ Urumampa-zhathodu (M)	Anardana, Anar Dadima, Dadimba, Sunila, Darmu, Daran, Darim, Pomegranate	*Punica granatum* L.	Fruit rind	SPI-II
36.	Mullilavu (M)	Pashadbhed	*Bergenia ligulata* (Wall) Engl.	Rhizome	Sharma & Sharma,1979
37.	*Munja* (M)	Gejeru,Ganikarnika, Arani, Ganiyari	*Premna serratifolia* Linn.	Stem	George, *et al.* 2006
38.	Murunkaip-pattai (T) / Muringa (M)	Sabinjana, Sabunjana	*Moringa oleifera* Lam.	Stem bark	SPI-II
39.	Muthanga (M)	Nagarmotha, Motha, Musta, Varida, Khana, Kuruvinda	*Cyperus rotundus* var. *centiflorus* C.B.Clarke	Rhizome	API- III
40.	Nagapoovu (M)	Nagkesara, Narmushk, Nageshor, Nahor	*Mesua ferrea* L.	Flower	Shome, Mehrotra and Sharma,1982
41.	Neermarthu (M)	Arjuna, Arjan, Dhanvi, Indradrum, Indra Vriksha	*Terminalia arjuna* (Roxb.ex DC.) Wight &Arn.	Stem bark	SPI-I
42.	Nelli, Nellikka (M)	Amla, Amalaki, Dhatri, Vayastha, Amritaphala	*Phyllanthus emblica* Linn	Dried fruit	UPI-I
43.	Njerinjil (M)	Gokhru, Nerinnil, Bahukantaka, Acuvacattiram, Chirupalleru	*Tribulus terrestris* L.	Fruit	SPI-I
44.	Orila (M)	Salwanpachang, Shalparni (Sarivan), Vidarigandha	*Desmodium gangeticum* (L.) DC.	Root	API- VI
45.	Pachotty (M)	Lodha	*Symplocos racemosa* Roxb.	Stem bark	API- I

S. No.	Trade Name in South India	Other Trade Names	Botanical species	Morpho-logical Part	Reference
46.	Padavalam (M)	Peipudal, Patola, Parwal	*Trichosanthes cucumerina* L.	Whole plant	Sandhya, Chandra-sekhar, David and Rao,2010
47.	Pananeer (M)	Satapatrika, Gul-e-Surkh	*Rosa centifolia* L.	Petal	API- III
48.	Pavakka / Kaipakka (M)	Karela, Kandula, Karakantaki, Bitter gourd, Carella fruit	*Momordica charantia* L.	Fruit	API- II
49.	Perarattai (T)	Peravathai, Perarattai, Kulanjan, Koracha, Malaya vacha, Greater galangal	*Alpinia galangal* (L.) Sw.	Rhizome	SPI-I
50.	Poolamaram (M)	Abresham	*Bombyx mori* Linn.	Silk Cocoon	UPI-VI
51.	Pushkara-moolam (M)	Pushkarmool	*Inula racemosa* Hook.f.	Root	API- IV
52.	Rakthachan-danam (M)	Raktachandan, Sandal Surkh	*Pterocarpus santalinum* L.f.	Heart wood	SPI-II
53.	Ramacham (M)	Vettiver, Usira, Vettiver, Khas	*Vetiveria zizanioides* var. *tonkinensis* A.Camus	Root	SPI-II
54.	Ratanjot (M)	Ratanjot	*Onosma hispidum*wall. ex. D. Don. syn. O. echioides C.B. Clarke non L.	Dried roots	UPI-III
55.	Sathakuppa (M)	Sowa, Soya, Dillseed, Kattucatakuppai, Sompa	*Anethum graveolens* L.	Fruit	UPI-V
56.	Sathavari (M)	Satavari, Shatawari	*Asparagus racemosus* Willd.	Root	SPI-II
57.	Thakaram (M)	Tagar, Farasiyun	*Valeriana wallichii* DC.	Rhizome	API- I
58.	Thamalpatram (M)	Tejpat, Tamal patri, Sajaz hindi	*Cinnamomum tamala* Ness and Eberm	Leaf	SPI-I

S. No.	Trade Name in South India	Other Trade Names	Botanical species	Morpho-logical Part	Reference
59.	Thamarayalli (M)	Tamarai malar	*Nelumbo nucifera* var. *lutea* (Willd.) Kuntze.	Stamen	SPI-I
60.	*Thannikka* (M)	Bhibhitaki,Tanrikkai, Balela, Bibhitaka	*Terminalia belerica* Roxb.	Fruit	SPI-I
61.	*Thazhuthama* (M)	Mukkirattaicca-mulam,Mookkirattai, Punarnava, Raktapunarnava	*Boerhaavia diffusa* L.	Root	API- III
62.	*Thippali* (M)	Filfil Daraz,Pippali	*Piper longum* L.	Fruit	SPI-I
63.	Trikolpakonna (M)	Danthal Tambacoo	*Operculina turpethum* (L.) Silva Manso	Root	API- III
64.	Uluva (M)	Methi, Hulba Chandika, Jyothi, Methika, Pithabeeja, Vdhini, Fenugreek	*Trigonella foenum-graecum* L.	Seed	SPI-I
65.	Vayambhu (T)	Vekhand, Bachal, Vacha, Waj-e-Turki, Bare, Baryan, Boiye, Boi, Halbou, Bach, Sweetflag, Camlamus root	*Acorus calamus* L.	Rhizome	API- II
66.	Vazhuthina (M)	Vartaku, Valuthalai	*Solanum melongena* L.	Root	Narayana, and Kolammal, 1962
67.	Veluthulli (M)	Akadiyalahsun, ek-kali lahsun, Ek-pothiyalahsun	*Allium ampeloprasum* Linn.	Bulb	Bedi, 2005
68.	White kidney bean	Soyabean	*Glycine max* (L.) Merr.	Seed	HPI-VI

Abbreviations: API-Ayurvedic Pharmacopoeia of India, SPI-Siddha Pharmacopoeia of India, UPI-Unani Pharmacopoeia of India, HPI-Homoeopathic Pharmacopoeia of India, IP Pharmacopoeia of India, QSIMP- Quality Standards of Indian Medicinal Plants, M-Malayalam-Tamil.



Figure-B



Figure-B Contd...



Figure-B Contd...



Figure-B Contd...



Fig B (1-68): 1. Adalodakam; 2. Amukkuram; 3. *Aratha*; 4. Banafshapatti; 5. Chakkarakolli; 6. Cheenapavu; 7. Chukku; 8. Elam; 9. Elavargam; 10. *Erattimadhuram*; 11. Ezhilampaala; 12. Gramboo; 13. Jatamanji; 14. Jeerakam; 15. Kacholam; 16. *Kadukka*; 17. Kanhiram; 18. Kanikonna; 19. Karimjeerakam; 20. Karivelam/ Karivelakam; 21. Karuvelam; 22. Kattuvizhalari; 23. Katukurokini; 24. Kiriyatha; 25. Kothambalayari; 26. Kottam; 27. Kunthirikkam; 28. Kurumulaku; 29. Kurunthotti; 30. Kutakappala/ Kutakapalayari; 31. Mahanikizhangu; 32. Manhal; 33. Maramanjal; 34. Masikka; 35. Mathalathodu/ Urumampazhathodu; 36. Mullilavu; 37. *Munja*; 38. Murunkaippattai/ Muringa; 39. Muthanga; 40. Nagapoovu; 41. Neermarthu; 42. Nelli; Nellikka; 43. Njerinjil; 44. Orila; 45. Pachotty; 46. Padavalam; 47. Pananeer; 48. Pavakka /Kaipakka; 49. Perarattai; 50. Poolamaram; 51. Pushkaramoolam; 52. Rakthachandanam; 53. Ramacham; 54. Ratanjot; 55. Sathakuppa; 56. Sathavari; 57. Thakaram; 58. Thamalpatram; 59. Thamarayalli; 60. *Thannikka*; 61. *Thazhuthama*; 62. *Thippali*; 63. Trikolpakonna; 64. Uluva; 65. Vayambhu; 66. Vazhuthina; 67. Veluthulli and 68. White kidney bean

Conclusion

There are number of herbal drugs reported to be adulterated or substituted in the trade (Dutt and Sharma, 2010; Sharma *et al.,* 2010, 2011; Sharma and Dutt, 2010). It may be intentional as malpractice or unintentional. The adulteration took place by replacement of specified species with allied species or different genera, admixing with degraded material or other species etc. The adulteration in herbal drugs occur due to collection of herbal drugs by unskilled gatherers, scarcity of plant species in the collection area, higher price value of the drug in the market, confusion in vernacular names and other reasons. Herbal drugs of root and bark origin are found to be adulterated more often.

The incidence of adulteration or substitution can be curbed by proper identification of herbal drugs at the time of collection and prior to herbal drug formulations. The identification of herbal drugs not only prevent use of adulterated or substituted material but it is also a prime requirement to assure the quality of manufactured herbal medicine. Quality of herbal drugs is not only confined to their identification, but freedom from foreign matter, biological (microbial or animal) and non-biological (heavy metals, pesticide residue) contaminants are also equally important for ensuring safety and efficacy of medicine formulated with these herbal drugs as ingredient.

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A Preliminary Study of Histamine Level in Vitiligo Patients

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Abstract

This preliminary study aims to add to the concept of stress participation in vitiliginous condition. For this, histamine level in the blood of vitiliginous patients was determined and the values were compared with that of the control. The results demonstrated that there occurs a significant increase in histamine level in patients whose vitiliginous lesions have just started.

Keywords: Vitiligo, Histamine, Stress.

Introduction

Though histamine is a natural constituent of the body, it is liberated from the cells of the skin by noxious stimuli including antigen-antibody reaction. Mast cells that liberate the histamine are present in the connective tissue throughout the body except the brain. They contain specialized Lysosomal granules which are composed of complex macro molecules of many biologically active substances formed by intra cellular synthesis (Alture, 1978; Elenkov and Chrusos, 2006). In antigen induced hypersensitivity reaction, the mast cells function as the effectors cell armed with an arsenal of mediators and with the ability to synthesize additional biological agents, which when released in response to the appropriate stimulus induce dramatic change in surrounding target tissues (Wasserman, 1979). Actually, mast cell degranulation may have generalized damaging consequences. Panja (1985) has assumed it previously that hypersensitive condition in the body prevails during conditions of amelanosis. As an immunologic basis of vitiligo persists, it was therefore of our interest, to compare the histamine level in the blood of vitiliginous patients with normal subjects to find whether there is any relationship between histamine secretion under vitiligenous conditions, in different human subjects.

Methodology

The study was conducted at the department of Dermatology, Calcutta Medical College & Hospital, Calcutta in the OPD of Prof. R.K. Panja during 16.12.1983 to 15.12.1988. Human subjects were divided into three groups of 30 subjects each. Patients suffering from vitiligo were included in test group while normal subjects were allocated to control group. Thus group I served as control group while group II & III served as test groups. Group II included the patients with early symptoms of vitiligo and the group III included the cases with extensive symptoms of vitiligo. The values were analyzed statistically using the Student's

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't' test. Care was taken that no subject is having any other systemic disease. Blood samples were collected from the two groups and the histamine level was determined. The findings of the two groups were compared with each other to find the difference, if any.

Extraction of histamine from blood

5 ml of blood was added directly to 10 ml of 10% Trichloroacetic acid. The mixture was shaken vigorously during and after the addition. The precipitate of blood was separated in a filtering flask under suction. The precipitate was washed 2-3 times with 10 ml of 5% trichloroacetic acid. Concentrated hydrochloric acid in ratios of 5 ml blood was added directly to the trichloroacetic acid filtrate. Then the extracts were boiled for 90 minutes over a flame on a sand – bath being careful to avoid charring. The volume of the extracts were maintained during boiling with acid by use of a reflux condenser and then heated to dryness in vacuum over a hot water bath maintained at 70°c. Final extracts were then prepared with distilled water.

Procedure for determination of histamine

It was determined by the method of Shore (1971). To a 2 ml aliquot of histamine solution in a small test tube, 0.4 ml 1N NaOH was added, followed by further addition of 0.1 ml of Ortho-thaldehyde reagent, after 4 min at room temperature. 0.2 ml of 3N hydrochloric acid was added. The tubes were shaken after each addition. Preparation of a standard curve of histamine was performed by the same way. The solutions were transferred to flurometer cuvets and the fluorescence were determined at 450 nm in a Perkin-Elmer Flurescence spectrophotometer at Regional Sophisticated Instrumentation Centre (RSIC), Bose Institute, Calcutta.

Observations and Result

The histamine concentration of vitiligo patients with extensive symptoms did not reveal any significant difference as compared to the control value.

The histamine contents of vitiligo patients with early symptoms of depigmentation showed significant increase in the histamine concentration when compared with group I and II.

Discussion

From the table 1, it appears that histamine level in blood are elevated in vitiligo patients where depigmentation is in a preliminary stage. It is well known that

Table 1: Determination of histamine level in the blood of patient's suffering from vitiligo along with controls

Subjects	Mean ± SE ng of Histamine/ml of blood
Control (Male only) (30)	63.50 ± 2.3 A
Patient's with early symptoms of Vitiligo (Male only) (30)	80.27 ± 5.4* B
Patient's with extensive Vitiligo (Male only) (30)	56.87 ± 3.3** C

n=30

* $p < 0.02$ (A vs B)

** $p < 0.001$ (B vs C)

under stress condition histamine secretion is increased (Nordlund *et al.*, 1982; Nakano and Suzuki, 1984; Boisseau, 1998). At the onset of disease stress factor may be of sufficient magnitude to stimulate the histamine release which after sometimes may reach a steady state condition when the vitiliginous condition is stabilized and the rate of increase of histamine release is decreased to a greater extent or slowed down. So, the increase of histamine activity lends further support to our idea that stress might be concomitant with depigmentation process. Further it has been elucidated by Srivastava and Jaju (1987) that exogenous administration of histamine activates histaminergic receptor in the hypothalamus which causes release of dopamine in pars intermediate and thereby inhibits Melanocyte Stimulating Hormone (MSH) release, thereby inhibiting pigmentation. But, the value of MSH on pigment dispession in human subjects has been questioned. It is also known that imidazole ring can accelerate the tyrosine aminotransferase activity. As histamine is built on imidazole fragment, so from structural consideration it is plausible that histamine may increase aminotransferase activity making facile conditions for the onset of vitiligo. Further hypersensitivity reactions during the depigmentation process may also cause production of lymphokines thereby inhibiting pigment formation. Further investigations are necessary, as other substances which have cytotoxic effects should be analyzed to reach a definite conclusion (Arck *et al.*, 2006). Histamine appears to play a significant role in the pathogensis of vitiligo characterized by faint hypopigmented patches with significant itching (Panja *et al.*, 2013).

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Ethnomedicinal Plants of Nilagiri and Hadagarh Forest Ranges of Odisha

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Abstract

Ethnobotanical field study was carried out to collect first-hand information on folk medicinal uses of plants by the rural and tribal people of Nilagiri forest range of district Balasore and Hadagarh forest range of district Keonjhar of Odisha state during August-September, 2014. A total of 32 ethnomedicinal plants species belonging to 31 genera and 23 families are reported. These are being used by local inhabitant to cure various ailments such as worms, asthma, body ache, boils, burn, constipation, cuts/wounds, diabetes, diarrhea, dysentery, eczema, fever, headache indigestion, joint pain, leucorrhoea, rickets, scabies, snake-bite, s*permatorrhoea, s*prain, stomachache, toothache etc. The information on traditional and folk medicinal plants were collected through interviews of knowledgeable rural and tribal people and local traditional healers '*Vaidyas'*. Folk medicinal plants are provided with botanical name, their family, local names, Unani name (if any), part(s) used, recipe and mode of administration in respect to different diseases and conditions. Scientific validation of all such folk medicinal species in the context of their curative properties/claims is re-stressed.

Keywords: Ethnomedicinal plants, Hadagarh and Nilagiri, Forest Ranges, Odisha, Folk claims.

Introduction

India has ancient history of use of plants in the indigenous systems of medicine such as Ayurveda, Unani and Siddha. According to the World Health Organization (WHO) about 80% of the people in the developing countries of the world rely on traditional medicine for their primary health care (Farnsworth *et al.*, 1985; Kumar *et al.*, 1998). Globally, about 85% of all medications are derived from plants for primary health care (Farnsworth, 1988). It is generally *estimated* that *over 6000 plants* in *India* are in *use* in *traditional*, *folk* and *herbal* (Huxley, 1984). Moreover use of medicinal plants is more beneficial than synthetic and modern medicines because of their efficacy, affordable cost and minimal side effects (Ullah *et al.*, 2013).

The state of Odisha has varied climatic regions with rich source of phytodiversity and many tribal communities who depend upon the local plant resources for different purposes especially to ure various ailments. As many as 62 different *tribal communities* constituting 22.6% of the total state population are reported and inhabit in different geographical areas of the state (Sahu *et al.*, 2010). *Nilagiri (21.46°N 86.77°E) forest range of Balasore district and* Hadagarh *(21.35°*N

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86.18°E) forest range of Keonjhar district is inhabitant by various tribes such as Gond, Munda, Mahali, Kolha, ho, Bhuiyan, Santal, Saora, Kora *etc.* The rural and tribal inhabitants of these forest ranges are deprived of modern health care system and they are dependent on traditional therapeutic methods to meet their primary health care needs. *They collect the different part(s) of the plants to cure various diseases.* The local inhabitants have vast knowledge about medicinal uses of plants and this knowledge is mostly undocumented so far and transmitted orally from generation to generation. Many important species that might lead to drug discovery may be lost in absence of proper documentation. Consequently loss of this potentially valuable knowledge will ultimately affect the whole society. Therefore, there is an urgent need to study the diversity, distribution and documentation of the traditional knowledge as a whole before it is lost forever. The documentation will not only be useful to preserve the valuable knowledge for future, but will also help to open new vistas of research to discover new drugs of natural origin which are much safe, less costly and with minimum or no side-effects. The present study is based on this rationale and provides first-hand information on some 32 folk medicinal species from the study area.

Material and Method

Field surveys were carried out during August-September, 2014 to collect first-hand data on ethnomedicinal plants from the study area. Information on the local use of medicinal plants for the treatment of various diseases was collected through semi-structured interviews with knowledgeable rural and tribal communities and the herbal healer called '*Vaidyas*'. Standard procedures including recording of field data in field books were adopted for collection, preservation and identification of the specimens (Jain and Rao, 1977). Plant specimen collected from field with their local names were identified with the help of Flora of Orissa (Saxena & Brahmam, 1996) and Botany of Bihar & Orissa (Haines, 1921-25) and confirmed with the authenticated herbarium specimens of Survey of Medicinal Plants Unit (SMPU) of Regional Research Institute of Unani Medicine (RRIUM), Bhadrak, Odisha. The botanical specimens have been deposited in the herbarium for future reference.

Enumeration

Acalypha indica L. (Euphorbiaceae); Local name: Kounsia: Unani name: Kuppi; Locality: Hathgarh-9992; Part used: Leaf; Ethnomedicinal uses: Leaves grinded into paste with water and applied topically on cut/wounds and scabies.

Achyranthes aspera L. (Amaranthaceae); Local name: Cheetki; Unani name: Vazanun; Locality: Inderpur-9980; Part used: Root; Ethnomedicinal uses:

Crushed roots (ca. 10 gm) are mixed with water (ca 100 m.) and given twice in a day to cure dysentery.

Aerva lanata (L.) Juss. Ex Schult. (Amaranthaceae); Local name: Jastimadhu; Unani Name: Biseributi; Locality: Hathgarh-9990, Bitusahi-9947; Part used: Root; Ethnomedicinal uses: Root decoction is given twice a day to treat diarrhea. Decoction of leaves is used to treat s*permatorrhoea.*

Alternanthera sessilis (L.) R. Br. *ex* DC. (Amaranthaceae); Local name: Modranga; Locality: Chhenapati-9969; Part used: Aerial part; Ethnomedicinal uses: The aerial part is consumed as vegetable to improve digestion.

Biophytum sensitivum (L.) DC (Oxalidaceae); Local name: Lajukoli; Locality: Panchlingeswar-9966; Part used: Leaf; Ethnomedicinal uses: One teaspoonful leaves juice is taken twice a day to treat diabetes.

Cassia fistula L. (Caesalpiniaceae); Local name: Sunari; Unani name: Amaltas; Locality: Chhenapati-9974; Part used: Stem; Ethnomedicinal uses: Stem is boiled in a glass of water till half glass is left. The decoction is used for leucorrhea for 20 days.

Clerodendrum indicum (L.) Kuntze. (Verbenaceae); Local name: Kanji Kanji; Locality: Chhenapati-9972; Part used: Root; Ethnomedicinal use: Roots are dried in shade and grinded into powder. Half table spoon of powder is taken for asthma twice in a day.

Alternanthera sessilis (J.Koenig) Sm. (Zingiberaceae); Local name: Ban-maka; Locality: Hathgarh-9989; Part used: Rhizome; Ethnomedicinal use: Decoction of rhizome is used to treat fever and to expel intestinal worms.

Crotalaria pallida Ait. syn. *C. stricta* DC. (Fabaceae); Local name: Nirmisi; Locality: Chhenapati-9975; Part used: Root; Ethnomedicinal use: Root paste is used as antiseptic on cuts/wounds to check bleeding.

Croton bonplandianus Baill. syn. *Croton sparsiflorus* Morong. (Euphorbiaceae); Local name: Vanmirichh or Methi; Locality: Chhenapati-9971; Part used: Latex of plant and leaf; Ethnomedicinal use: Latex of plant is used for eczema. Paste of leaves is used on cut/wounds for quick healing.

Cuscuta reflexa Roxb. (Cuscutaceae); Local name: Nirmuli; Unani name: Aftimoon; Locality: Bitusahi-9951; Part used: Stem; Ethnomedicinal use: Plant paste prepared in mustard oil is applied warm on sprain to get relief from pain.

Dioscorea bulbifera L. (Dioscoreaceae); Local name: Pita Alu; Locality: Bitusahi-9949; Part used: Rhizome; Ethnomedicinal use: Tuber powder is given on the early morning with warm water as laxative to treat constipation. Rhizome cooked and eaten as vegetable.

Diospyros montana Roxb. (Ebenaceae); Local name: Kadurki; Locality: Bitusahi-9946; Part used: Ripe fruits; Ethnomedicinal use: Ripe fruits are eaten raw to treat dysentery.

Erythrina variegata L. (Fabaceae); Local name: Pharadn; Locality: Bitusahi-9943; Part used: Leaf; Ethnomeidicnal use: 2-3 ml leaf juice with required quantity of turmeric is given to children to expel worms.

Glycosmis pentaphylla (Retz.) DC. (Rutaceae); Local name: Mohri; Locality: Hathgarh-9991; Part used: Leaf; Ethnomedicinal use: Leaves are made into paste with ghee and used to cure boils.

Gymnema sylvestre (Retz.) R. Br. ex Schult. (Apocynaceae); Local name: Gudmari; Unani name: Gudmar; Locality: Bitusahi-9950; Part used: Leaf; Ethnomedicinal use: 5-10 gm leaves powder is taken with water to treat diabetes.

Helecteres isora L. (Sterculiaceae); Local name: Mudi, Dauragundi; Unani name: Marorphali; Locality: Panchlingeswar-9958, Hathgarh-9988; Part used: Pod, Root; Ethnomedicinal use: A handful of fruits boiled in mustard oil, cooled and filtered. Resultant medicated oil is massaged gently on affected legs of children to cure rickets. Decoction of root (ca. 50 ml) is used to cure constipation.

Holarrhena pubescens (Buch.-Ham.) Wall. ex G. Don (Apocynaceae); Local name: Kurin; Unani name: Inderjo Talkh; Locality: Bitusahi-9941; Part used: Stem bark; Ethnomeidicnal use: Powdered stem bark is taken with sufficient water to check dysentery.

Jatropha curcas L. (Euphorbiaceae); Local name: Jahaji; Unani name: Baghrendah; Locality: Bitusahi-9944; Part used: Latex; Ethnomedicinal use: Latex is applied fresh on minor cuts.

Jatropha gossypifolia L. (Euphorbiaceae); Local name: Laljahaji; Locality: Bitusahi-9942; Part used: Plant sap; Ethnomedicinal use: Plant sap is applied on gums to treat toothache.

Kalanchoe pinnata (L.) Pers. (Crassulaceae); Local name: Amarpoi; Unani name: Zakhm-e-Hayat; Locality: Bitusahi-9954; Part used: Leaf; Ethnomedicinal use: Leaves paste is applied on forehead to get relief from headache.

Leucas cephalotes (Roth.) Spreng.syn *Phlomis cephalotes* Roth. (Lamiaceae); Local name: Goeso; Unani name: Guma; Locality: Hathgarh-9994; Part used: Root, Leaf; Ethnomedicinal use: Root decoction in desired quantity is taken for snakebite. Paste of leaves is used for boils.

Lygodium flexuosum L. (Lygodiaceae); Local name: Mahajal; Locality: Panchlingeswar-9962; Part used: Root; Ethnomeidicnal use: Root decoction is given to treat Leucorrhoea.

Martynia annua L. (Martyniaceae); Local name: Baghnakhi; Unani name: Kala; Locality: Bichhua-9940; Part used: Leaf; Ethnomedicinal use: Leaves juice is applied locally on fresh cuts to check bleeding. Past of ripen fruit is used for scabies and burn.

Mimosa pudica L. (Mimmosaceae); Local name: Jau-gochho; Unani name: Lajjalu; Locality: Chhenapati-9973; Part used: Root; Ethnomedicinal use: Roots are made into paste with water and applied on wounds for quick healing.

Pavetta indica L. (Rubiaceae); Local name: Kuruma Gach; Locality: Panchlingeswar-9961; Part used: Leaf; Ethnomedicinal use: Leaves decoction is given to treat body ache.

Rauvolfia tetraphylla L. (Apocynaceae); Local name: Patalgaruda; Locality: Podasul-9936; Part used: Root; Ethnomedicinal use: 3-5 gm powdered root is taken 2-3 times a day with water to treat stomachache. Root extract (30 ml) is used for diarrhea and dysentery.

Tephrosia purpurea (L.) Pers. (Fabaceae); Local name: Kulthia; Unani name: Sarphoka; Locality: Podasul-9935; Part used: Root; Ethnomedicinal use: Half teaspoonful powdered root is taken twice a day with water to get relief from stomachache.

Terminalia bellirica (Gaertn.) Roxb. (Combretaceae); Local name; Baheda; Unani name: Balela; Locality: Hathgarh-9981; Part used: Stem bark; Ethnomedicinal use: Decoction (ca. 20 ml) of stem bark is drunk to get relief from diarrhea.

Vanda tessellata (Roxb.) Hook. *ex* G. Don. (Orchidaceae); Local name: Modang; Unani name: Banda; Locality: Hathgarh-9982; Part used: Leaf; Ethnomedicinal use: Paste of leaves is used for rheumatic pain.

Vitex negundo L. (Verbenaceae); Local name: Begunia; Unani name: Sambhalu; Locality: Podasul-9937; Part used: Leaves; Ethnomedicinal use: 30 ml leaves decoction is given twice a day for 21 days to treat vatrogo (Joint Pain). Leaves are used as insecticide to stored grains.

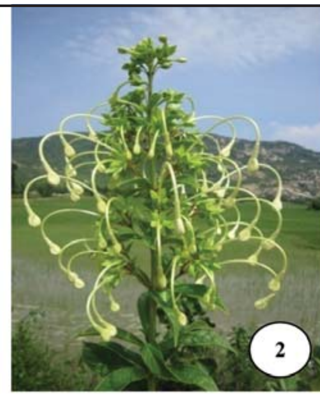
Ziziphus mauritiana Lamk. (Rhamnaceae); Local name: Ber; Unani name: Barokoli; Locality: Inderpur-9976; Part used: Stem bark; Ethnomedicinal use: 30 ml of stem bark decoction is given twice in a day for checking dysentery.

Results and Discussion

Forest resources of the study area fulfill the primary needs of the local inhabitant and various plants species are used for herbal preparations (Fig. 1-9). These medicinal plants play a significant role in meeting their primary healthcare needs. In the present study 32 medicinal plants species belonging to 31 genera and 23



Aerva lanata (L.) Juss. Ex Schult.



Clerodendrum indicum (L.) Kuntze.



Helecteres isora L.



Holarrhena pubescens (Buch.-Ham.) Wall. ex G. Don



Jatropha curcas L.



Martynia annua L



Mimosa pudica L.



Tephrosia purpurea (L.) Pers.



Vanda tessellata (Roxb.) Hook. *ex* G. Don.

Figures (1-9): Some ethnomedicnal plants of study area

families are reported (Fig. 10). The leaves (twelve spp.) were the most frequently used part followed by root (ten spp.), stem bark (three spp.), latex, rhizome, stem (two species each), aerial part, plant sap, pod, ripe fruits (one spp. each) (Fig. 11&12). Mode of administration of the herbal preparation included oral administration in the form of powder, decoction, infusion, extraction and topically

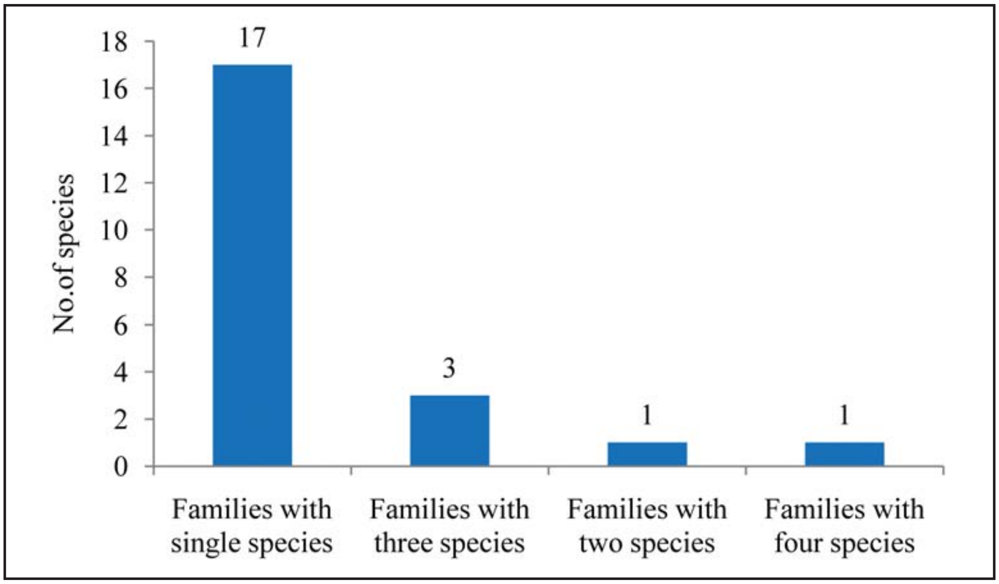


Figure 10: Distribution of families in the study area based on number of species

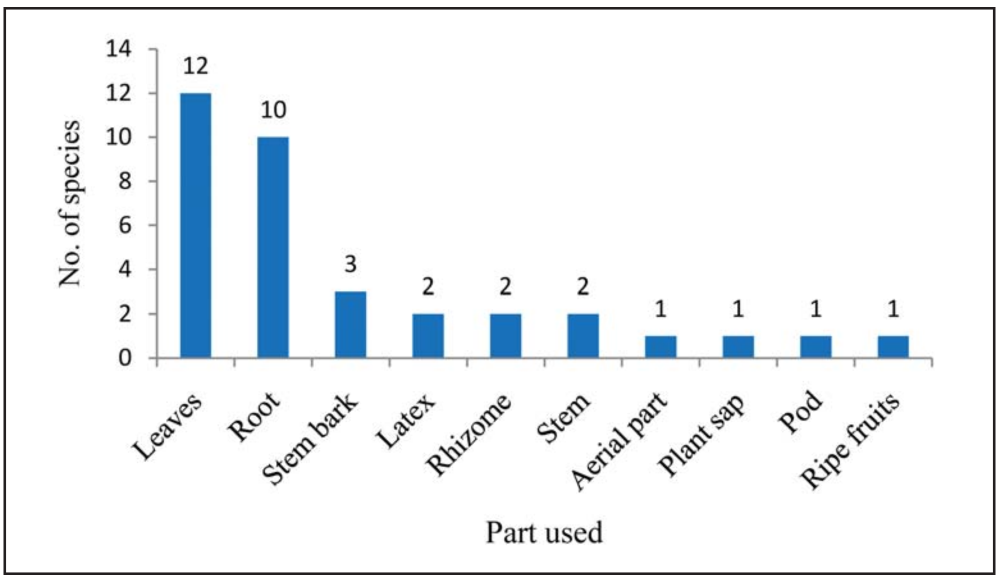


Figure 11: Different parts used in the study area for medicinal preparation

applied in form of paste for skin problem, headache and joint pain. To make powder, plant part is dried and grinded into fine powder. The decoction was prepared by boiling the plant parts in water until the volume of the water reduced to the minimum or required amount. However, extract of the plant part is prepared by straining well pounded fresh material. Some are consumed as raw and in cooked form to cure the disease. Most of the recipes of herbal plants were taken once or twice a day as a full dose, depending on age, health and types of ailment. Some major ailments such as diabetes, respiratory diseases, snakebite, skin diseases, stomach disease etc. are being effectively treated using traditional knowledge and locally available plant resources. Maximum eleven species are

used to treat to gastrointestinal disorders followed by skin disorders (eight species).

Most of the folk medicinal species reported from the study area have also been reported in previous studies (Ambasta, 1986; Aminudin *et al.*, 2013; Behera *et al.*, 2006; Behera *et al.*, 2008; Dhal *et al.*, 2014; Jain ,1991, Khare, 2007; Kirtikar & Basu, 1935; Mukesh *et al.*, 2011; Mukesh *et al.*, 2012; Mukesh *et al.*, 2014a, 2014b; Patra *et al.*, 2014; Rout *et al.*, 2009; Usha *et al.*, 2014), though their mode of administration, ingredients and part used are different. However the similar use of the plants reported in previous studies in different tribal communities is imperative and may be a trustworthy indication of their curative properties. Therefore, present study represents the contemporary use of folk medicinal plants from the study area. Furthermore, for most species, information on their biological activity and chemical constituents is available in the literature (Chandel *et al.*, 1996; Kirtikar and Basu, 1935). However, with the availability of recent scientific tools, there is a need to re-investigate all these folk medicinal species for their chemical constituents and pharmacological activity in an effort to discover new drugs of plant origin.

The observations made under the present investigation conclude that abundant indigenous knowledge on traditional medicine mainly involving the use of the natural plant resources plays a significant role in meeting the primary healthcare needs of the tribal people. They use locally available plant resources to cure various ailments which provide a cheaper and accessible alternative to the high cost pharmaceutical remedies. High dependability and strong belief of the local people on the curable properties of the available plants resources depicts their pharmaceutical potential. Thus, this study should be of great use from pharmaceuticals point of view which would provide baseline information for future research and biological resource management. Furthermore, the traditional medicines are impacted by the anthropogenic activities such as deforestation, habitat destruction, industrialization, unsustainable utilization, rapid socioeconomic and cultural changes. Therefore great concern should be taken for their conservation and sustainable use and utilization before their permanent depletion.

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Standardization of a Unani Drug Bisehri Booti (*Aerva lanata* Linn.)

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Abstract

Aerva lanata L. (Family: Amaranthaceae) known as 'Bisheri Booti' in Indian subcontinent, has been mentioned in few classical Unani literature and is used frequently by Hakeems of Western Uttar Pradesh in different urinary diseases. In Unani literatures it has found only fractional description where mainly organoleptic characters have been described. Till now Unani physicians have not adopted scientific methods for identification and standardization of a numbers of Unani drugs including Bisehri Booti (*A. lanata*). The present study was therefore undertaken to determine the preliminary physico-chemical and phytochemical characteristics of *A. lanata* on qualitative parameters in which it was found that alkaloids, amino acids, proteins, glycosides, saponins, tannins, sterol/terpenes, sugars and flavonoids were present, while phenol and resins were absent. A number of attributes such as solubility in alcohol (1.67%) and water (3.07%), pH at 1% (7.39) and 10% (6.36%), moisture content (4.2%), total ash value (7.30%), loss of weight on drying (5.92%), bulk density (0.33%), successive extractive values Petrolium ether (2.92%), Diethyl-ether (0.22%), Chloroform (0.38%), Acetone (0.27%), Alcohol (9.27%), Water (14.38%), non successive extractive values in Alcohol (11.98%) and water (12.69%) were recorded. These parameters may help to standardize the test drug.

Keywords: Bisehri Booti, Standardization, Physico-chemical and Phyto-chemical, Amaranthaceae, *Aerva lanata* Linn.

Introduction

Bisehri Booti (*Aerva lanata* Linn.; family *Amaranthaceae*) (Fig. 1) is relatively a less known drug of Unani Medicine which is used in Haematuria, Burning micturation, Albuminuria, Lithiasis and some other nephrological disorders by many Unani physicians especially in Western Uttar Pradesh (Afaq *et al.*, 1991; Savadi and Algavadi, 2009; Ragavendran *et al.*, 2012). Although it has not been mentioned in important books of Unani medicine, however a physician of twentieth century Hm. Abdul Qadir (Qadir, 1930) has mentioned this drug in his book Mujarrabat-e-Qadri with necessary details. Bisehri Booti has been identified as *Aerva lanata* (Linn.) (Afaq *et al.*, 1991). Over a period of time it got reputation as an antilithiatic agent which was also effective in other ailments of kidney and prostate etc. (Afaq *et al.*, 1991; Afridi, 1992; Ahmad, 1994).

Bisehri Booti is a woody, prostrate or succulent perennial herb, 30-60 cm in height, native of Asia, Africa, and Australia. The plant is distributed throughout the hotter parts of India especially all over plains and hilly area up to 900 mts.

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It spreads in the state of Tamil Nadu, Andhra Pradesh and Karnataka, plains of Bengal from Deccan, Westward to Konkan, Madhya Pradesh and also in Punjab and Trai region of U.P. (Karnick, 1972; Kapoor, 1976). Flowering season is from Oct/ Nov to June (Patnaik, 1956; Trimen, 1974; Ahmad, 1994). Although, the whole plant is used to treat a number of diseases but the differential effects of different parts of the plant have also been described in Unani and Ayurvedic literature. It is also reported to be present in Sri Lanka, South Asia, Saudi Arabia, Egypt, tropical Africa, South Africa, Java and Philippines (Lakshami and Lethi, 2014).

The root is claimed to be demulcent diuretic and useful in strangury in Ayurveda, The roots are used in the treatment of headache. It is valued for cough, also as a vermifuge for children (Dymock, 1890; Kirtiker and Basu, 1987). The plant is an anthelmintic and demulcent and is used in lithiasis. It is also regarded as useful medicine for cough, sore throat, indigestion, wounds and for diabetes. A decoction of the plant is considered an efficacious diuretic and is considered useful in catarrh of bladder. The plant is used to cure diarrhea, cholera and dysentery. The root is diuretic, demulcent, tonic and is given to pregnant women. The root and flower are used to cure headache. The flowers are used in gonorrhea and for removal of kidney stones (Nadkarni, 1976; Chopra *et al.*, 1956; Gupta and Tandon, 2004). The herb is used in malaria and skin disease. In piles, it is given with black pepper and milk (Afaq *et al.*, 1991; Anonymous, 2000). The plant is useful to treat boils, cephalgia, cough, strangury and lithiasis (Sala, 1993).

The description of the plant in the literature of traditional medicines and its age-old practice by the physician of Unani and Ayurvedic medicine to treat a number of diseases specially the kidney diseases successfully, indicate that it is an important medicinal plant which has wide therapeutic potential. Further, in a recent study it has been reported to possess significant nephroprotective effect against chemically induced nephrotoxicity (Ahmad, 1994). However, the drug has probably not been standardized on scientific parameters as sufficient data is not available to determine its identity and quality. In view of the above therefore physico-chemical and phyto-chemical study of this drug was carried out in order to fix its standard.

Material and Method

Collection of Plant material

The whole plant of Bisehri booti (*Aerva lanata* Linn) was collected from the premises of Ajmal Khan Tibbiya College, Aligarh Muslim University, Aligarh. Its identity was confirmed by the Pharmacognosy section of the department of Ilmul

Advia, AKTC, AMU Aligarh. A Voucher specimen (SC-0140/13S) of the plant material has been deposited in the Herbarium and Museum of department of Ilmul Advia, Faculty of Unani Medicine, AMU, Aligarh, for record and future reference.

Parameters of Standardization

(i) The organoleptic characters including colour, smell, texture, taste, appearance were noted carefully.

(ii) The dried powder of whole plant was used for chemical analysis. Various physico-chemical studies such as total ash, acid insoluble ash, water soluble ash, alcohol and water soluble matter, bulk density, moisture content, successive extractive values using soxhlet extraction method, and pH studies were carried out as per guidelines of WHO (Anonymous, 1998, 2008 ; Afaq *et al*., 1994; Jenkins *et al*., 1967).

(iii) Qualitative analysis of the drug was conducted to identify the organic chemical constituents present in the drug (Overtone, 1963; Harborne, 1973).

(iv) The thin layer chromatographic analysis was conducted following Stahl (1969) and Harbone (1973) method on pre-coated silica gel 60F264 TLC plates. The plates were visualized in day light, in short UV and Long UV.

(v) Fluorescence analysis of the successive extract and powdered drug were studied under day light as well as in short UV and Long UV.

Observations

(a) Organoleptic characters: The powder of the plant Bisehri Booti was found to be light green with agreeable smell and astringent taste (Table 1).

(b) Physico-chemical constants: Different physicochemical constants determined using suitable measures. The values recorded have been presented in Table 2 & 3.

Table 1: Organoleptic Character of Bisehri Booti (*Aerva lanata* Linn)

Colour	Light green
Appearance	Powder
Texture	Fine
Taste	Astringent
Smell	Agreeable

Table 2: Physico-chemical constants of powder of Bisehri Booti (*Aerva lanata* Linn)

S.No.	Parameters	Percentage (w/v)*
1.	Ash value	
	Total ash	7.30
	Acid insoluble ash	1.40
	Water soluble ash	5.80
2.	Soluble Part	
	Ethanol soluble	1.67
	Aqueous soluble	2.77
3.	Successive Extractive Value	
	Pet. Ether	2.92
	Di-ethyl ether	0.22
	Chloroform	0.38
	Acetone	0.27
	Alcohol	9.27
	Aqueous	14.38
4.	Non Successive Extractive Values	
	Alcoholic	11.98
	Aqueous	12.69
5.	Moisture content	4.22
6.	Loss on Drying	5.92
7.	pH values	
	1% water solution	7.39
	10% water solution	6.36
8.	Bulk density	0.33

*Note: Values are average of three experiments

(c) Fluorescence studies of the powdered drug after its reaction with different chemical reagents and successive extract were studied under day light as well as in short UV and Long UV (Table 4 & 5 respectively).

(d) TLC profile of different extract in different solvents have been recorded in (Table 6 & Fig.2).

Discussion

Physico-chemical and Phyto-chemical standardization are considered most important tools of quality control of Unani drugs. The efficacy and potency of a

Table 3: Phyto-chemical constituents determined after the qualitative study of extract of Bisehri Booti

S.No.	Test	Test/ Reagent	Inference
1.	Alkaloid	Drgendorff's reagent Wagner's reagent Mayer's reagent	+ve +ve +ve
2.	Amino acid	Ninhydrin Solution	+ve
3.	Protein	Xanthproteic Test Biuret Test	+ve +ve
4.	Glycoside	NaOH Test	+ve
5.	Flavonoid	Mg ribbon and Dil. Hcl	+ve
6.	Phenol	Ferric Chloride Test	-ve
7.	Resin	Acetic Anhydride Test	-ve
8.	Sterol/ Terpene	Hosse's Reaction Test Moleschott's Reaction	+ve +ve
9.	Sugar	Molisch Test Benedict Test	+ve +ve
10.	Tannin	Ferric Chloride Test	+ve
11.	Saponin	Honey Comb Frothing Test	+ve

Indications: (-ve) Absence and (+ve) Presence of constituent



Fig. 1: Bisehri Booti (*Aerva lanata* Linn) Plant

Table 4: Fluorescence analysis of Bisehri Booti powder with different chemical reagents

S. No.	Powdered drug+ Chemical Reagents	Day light	UV short	UV long
1.	Powdered drug + Conc. HNO_3	Dark Brown	Dark Green	Black
2.	Powdered drug + Conc. HCl	Dark Brown	Dark Green	Black
3.	Powdered drug + Conc. H_2SO_4	Dark Red	Black	Black
4.	Powdered drug + 2% Iodine solution	Dark Red	Blackish Green	Black
5.	Powdered drug + Glacial Acetic Acid	Cherry Red	Black	Black
6.	Powdered drug +NaOH (10%)	Light Green	Dark Green	Green
7.	Powdered drug + Dil. HNO_3	Light Green	Green	Dark Green
8.	Powdered drug + Dil. H_2SO_4	Green	Green	Black
9.	Powdered drug + Dil. Hcl	Light Green	Green	Blackish Green
10.	Powdered drug +Dragendorff's reagent	Brown	Dark Green	Black
11.	Powdered drug + Wagner's reagent	Dark Brown	Green	Black
12.	Powdered drug + Benedict' reagents	Light Green	Green	Grey
13.	Powdered drug + KOH (10%) Methanolic	Light Green	Light Green	Green
14.	Powdered drug + CuSO4 (5%)	Greenish White	Light Green	Cherry Green
15.	Powdered drug +Ninhydrin (2%) in Acetone	Light Green	Green	Grey
16.	Powdered drug + Picric Acid	Greenish Yellow	Green	Dark Green
17.	Powdered drug + Lead Acetate (5%)	White	Green	Dark Green

Table 5: Fluorescence analysis of Bisehri Booti extract in different lights

S.No.	Extract	Day Light	UV Short	UV Long
1.	Pet. Ether	Green	Green	Purple
2.	Di-ethyl ether	Light Green	Dark Green	Greenish
3.	Chloroform	Grey	Dark Green	Dark Green
4.	Acetone	Light Yellow	Light Green	Dark Green
5.	Alcohol	Dark Brown	Dark Green	Dark Brown
6.	Aqueous	Cherry Brown	Black	Black

Table 6: Thin Layer Chromatography profile

Treatment	Mobile Phase	Visible	No. of Spots	R_f values of the spots
Petroleum Ether Extract				
Day light UV Short UV Long	(i) P. Ether: Diethyl Ether (4:1)	5 5 5		0.42,0.47,0.69,0.90,0.95. 0.42,0.47,0.69,0.90,0.95. 0.42,0.47,0.69,0.90,0.95.
Day light UV Short UV Long	(ii) Chloroform: Methanol (1:1)	3 3 3		0.537,0.629,0.857. 0.537,0.629,0.857. 0.537,0.629,0.857.
Day light UV Short UV Long	(iii) Chloroform: Acetic Acid (4:1)	4 4 4		0.763,0.833,0.944,0.972. 0.763,0.833,0.944,0.972. 0.763,0.833,0.944,0.972.
Chloroform Extract				
Day light UV Short UV Long	(i) P. Ether: Diethyl Ether (4:1)	4 4 4		0.047,0.142,0.380,0.476. 0.047,0.142,0.380,0.476. 0.047,0.142,0.380,0.476.
Day light UV Short UV Long	(ii) Chloroform: Methanol (1:1)	3 3 3		0.081,0.754,0.803. 0.081,0.754,0.803. 0.081,0.754,0.803
Day light UV Short UV Long	(iii) Chloroform: Acetic Acid (4:1)	3 3 3		0.853,0.946,0.986. 0.853,0.946,0.986. 0.853,0.946,0.986.

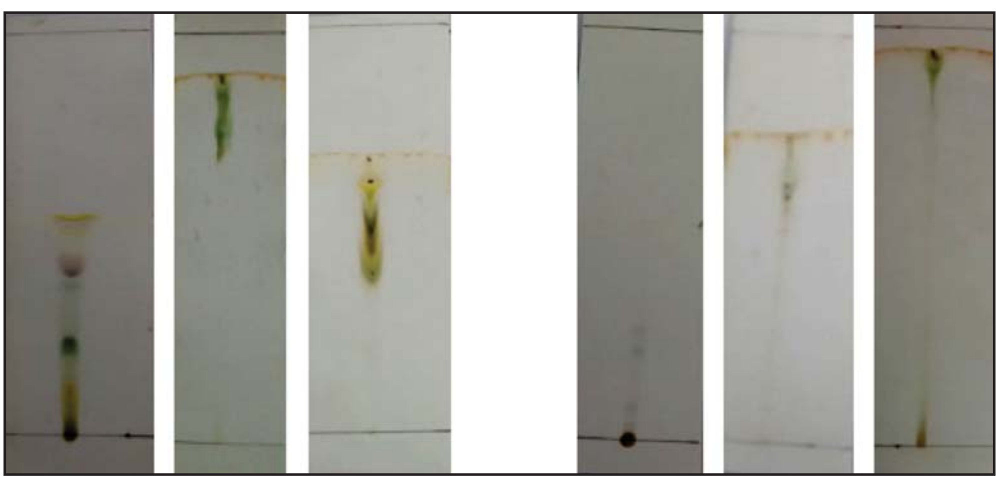


Fig. 2.1: TLC of Pet. Ether Extract Fig.2.2: TLC of Chloroform Extract

drug depends upon its physico-chemical as well as on Phyto-chemical properties, therefore, determination of Physico-chemical characters of the drugs for the purpose of identification is necessary so that the drug can be used successfully for its described pharmacological effect. Since the chemical constituent present in plant drugs vary not only plant to plant but also among different samples of same species, depending upon soil condition, atmosphere, collection, storage, and drying conditions of the sample drugs therefore the identity and quality of the plant intended to be used in the management of diseases must be ensured. It will help in maintaining the objectivity of the pharmacological activity and its therapeutic use.

Since the present study presents one of the earliest reports on the standardization of Bisehri Booti therefore, the findings recorded in the respect of various organoleptic, physic-chemical and phyto-chemical parameters set the standard of its identity and quality. It can be used by physicians, pharmacists and manufacturer as a reference. The TLC profile indicated that the test drug possesses 5 different constituent which may be responsible for the biological activity of the plant in different diseases. It warrants therefore, that further quantitative and analytical studies should be conducted to find out the main constituent or the combination of constituent of pharmacological and therapeutic utility.

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Botanical and Physico-chemical Standardization of Habb-e-Harsinghar – A Unani Formulation

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Abstract

In the past few decades there is growing sense of awareness among the developing world about the importance of traditional systems of medicine such as Unani, Ayurveda and Siddha for maintaining health without side effects. Following this emerged research activities like quality standardization of traditional medicines and development of scientific methods for the manufacture of quality medicine are on the rise. In view of this botanical and physico-chemical standardization of Habb-e-Harsinghar – A Unani formulation has been carried out. Present paper deals with the proper authentication, taxonomic identification, organoleptic characters, ingredient identification, physico-chemical values and chromatographic profile of the drug studied so as to set its standards for quality assurance for global marketing.

Keywords: Standardization, Quality assurance, Habb-e-Harsinghar

Introduction

Huboob (pills) are small, round and uniformly shaped medicinal preparations used in Unani system of medicine (Anonymous, 2006). Being mulaiyin (aperient) in action Habb-e-Harsinghar is frequently prescribed by Unani physicians in the treatment of bawaseerdamiya (bleeding piles) and bawaseeramya (blind piles). According to the formula composition, this drug contains two plant ingredients i.e. maghz-e-tukhm-e-Harsinghar and filfil siyah (Anonymous, 2007).

Maghz-e-Tukhm-e-Harsinghar are the seeds of *Nyctanthes arbor-tristis* Linn. of family Oleaceae. It is considered as cholagogue, anthelmintic and laxative in action. In Unani system of medicine it is useful in piles .Filfil siyah are the berries of *Piper nigrum* Linn. of family Piperaceae . It is acrid , pungent and hot. In unani system of medicine being hot 2<”and dry 2<” it is considered as carminative, aphrodisiac, purgative, alexipharmic, removes balgham. (Anonymous, 1966, 1969; Chopra *et al.*, 1969; Kirtikar and Basu, 1988; Nadkarni, 1986)

In order to lay down the standards for manufacturing the quality medicine, the drug was prepared in three different batches at laboratory scale. Present paper describes the salient features of preparation, micoscopical characters, physico-chemical and thin layer chromatography data of Habb-e-Harsinghar.

Material and Method

In order to develop the quality medicine with maximum therapeutic potential all the ingredients were procured from the local raw drug dealers, New Delhi. After

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proper identification of each ingredient (Anonymous, 2007; 2008), Habb-e-Harsinghar was prepared as per following formulation composition :-

1.	Maghz-e-Tukhm-e-Harsinghar	1.5 kg
2.	Filfil siyah	500g

Microscopic Observation

Few pills were broken into fine powder and examined microscopically after staining with different reagents like saffranine, iodine solution, ferric chloride solution etc. and mounted with glycerine. (Johansan, 1940; Iyengar, 1997; Trease and Evans, 1983; Wallis, 1969). The representative photographs were taken from the computer with microscopic attachment .

Chemical Analysis

All the prepared batch samples were subjected for chemical analysis. Physico-chemical studies like total ash, acid insoluble ash, solubility in alcohol and water, loss on drying at 105<”C were carried out(Anonymous, 1998) .

Thin Layer Chromatography

Preparation of extract for TLC

5g. powder drug was extracted in 60 ml. of absolute alcohol under reflux of water bath for 10 minutes and filtered. Further the filterate was concentrated upto 4ml.and used for thin layer chromatography (Wagner, 1984).

Preparation Method

Both the ingredients were taken of pharmacopoeial quality. Cleaned, dried, powdered separately and sieved through a mesh no. 100. Then both the ingredients were mixed together and kneaded with water to make the dough. The sticks of dough were made and rolled between the fingers to make the pills of approx. 250 mg. size. The yield was 520 pills. Packed in tightly closed containers to protect from light and moisture. The formulation was prepared in three batches separately by the same method.

Results and Discussion

Habb-e-Harsinghar is a dark brown coloured solid pill with bitter taste and unspecific odour. The drug did not show any change or fungal growth when kept in a petri dish.

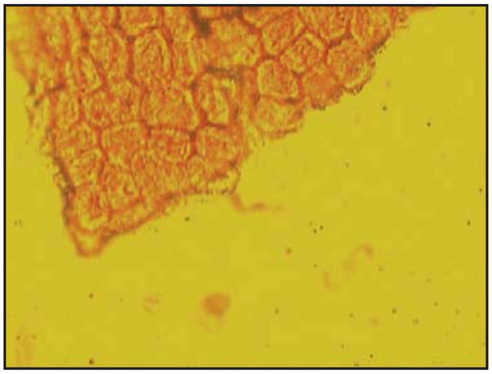


Fig. 1 X40 Sclereids in groups of *Piper nigrum* Linn.

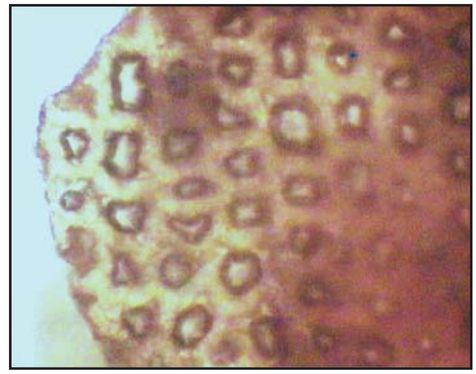


Fig. 2 x40 Sclereids in groups of *Piper nigrum* Linn.

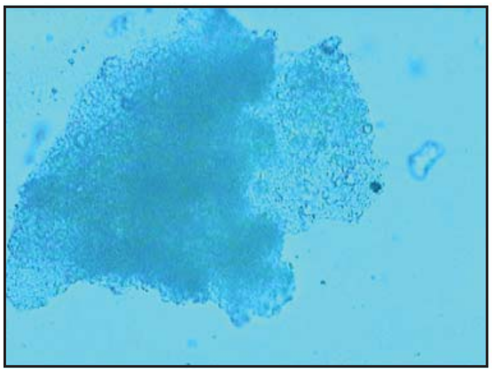


Fig. 3 X40 Parenchyma cells with starch grains of *P. nigrum* Linn.

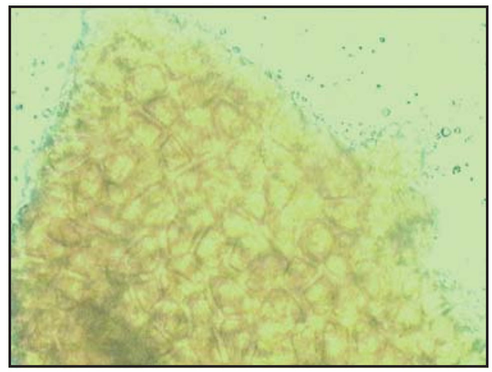


Fig. 4 x40 Epidermal cells in surface view of *Nyctanthes arbortristis* Linn.

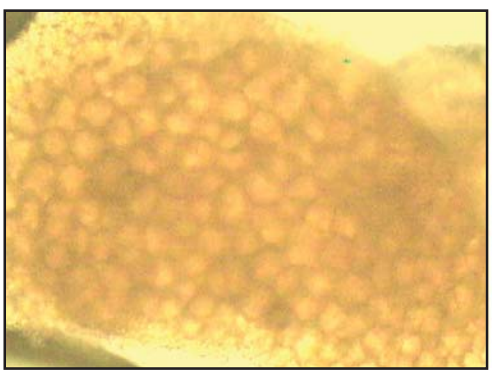


Fig. 5 X40 Palisade cells in surface view of *Nyctanthes arbor-tristis* Linn.



Fig. 6 x40 Palisade cells in sectional view of *Nyctanthes arbor-tristis* Linn.

Fig. 1-6: Microscopic Observations on Drug – Habb-e-Harsinghar

Microscopic Observation

On examination under the microscope Habb-e- Harsingharshows epidermal cells in surface view, palisade cells in surface and sectional view (Maghz–e-Tukhm-e-Harsinghar);groups of more or less iso-diametric or slightly elongated stone cells and parenchymatous cells filled with starch grains (Filfil siyah) (Fig. 1-6).

Chemical Analysis

Table 1: Physico- chemical data of the drug

S. No.	Parameters	Batch No.		Batch No.		Batch No.	
		I	Mean Value	II	Mean Value	III	Mean Value
Extractives							
1.	Alcohol soluble matter	18.00 19.20 20.80	19.30	16.80 16.80 17.40	17.00	15.20 17.60 18.40	17.07
2.	Water soluble matter	35.20 35.20 36.00	35.47	36.60 37.60 38.40	37.53	38.40 38.40 39.60	38.80
Ash Values							
1.	Total ash	3.75 4.00 4.00	3.92	4.05 4.10 4.25	4.13	3.25 3.50 3.55	3.43
2.	Acid insoluble ash	0.60 0.65 0.65	0.63	1.05 1.05 1.15	1.08	0.65 0.75 0.75	0.72
3.	Loss in wt. on drying	5.00 5.50 5.90	5.47	6.50 6.50 6.50	6.50	6.40 6.75 7.00	6.72
pH Values							
1.	1% aq. Sol.	4.23 4.60 4.61	4.48	4.46 4.54 4.60	4.53	4.46 4.51 4.53	4.50
2.	10% aq. Sol.	4.05 4.12 4.13	4.10	4.07 4.11 4.12	4.09	4.07 4.12 4.16	4.12

Thin Layer Chromatography Analysis

5 g. of powdereddrug was extracted with 60 ml. of ethanol under refluxing conditions on water bath for 10 minutes and filtered. The filtrate was concentrated up to 4 ml. The extract so obtained was applied on a pre-coated silica gel plate and the solvent system Toluene: Ethyl Acetate (90: 10) was used in developing chamber to develop it. The plate was dried and sprayed with Vanilin-Suplhuric acid reagent. The plate was again dried and kept in an oven for heating at 105p C for 10 minutes. Rf values of the spots are: 0.22; 0.31; 0.38; 0.45; 0.49; 0.53; 0.58; 0.63; 0.71; 0.84; 0.96.

Conclusion

Microscopic examination of the drug Habb-e-Harsinghar provides a key diagnostic histological characters that are helpful in establishing the identity of the genuine drug material. Physico-chemical parameters viz. ash values, extractive values and pH etc. of the studied drug serves as an important tool in maintaining the batch to batch consistency so as to bring a safe, efficacious and quality product for the global market.

Acknowledgement

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Antibacterial Screening of Karanjwa Seeds (*Caesalpinia bonducella* Roxb.): An Effective Unani Medicine for Infectious Diseases

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Abstract

Karanjwa (*Caesalpinia bonducella* Roxb.) has been reported in various Unani Classical books to be used in infectious diseases, but the study to confirm its efficacy scientifically is not done so far. Therefore, the present investigation was carried out to evaluate its antibacterial efficacy against various pathogenic bacterial strains by using Kirby Bauer's Disk Diffusion and Agar well method according to Clinical Laboratory Standard Institute (CLSI) Guidelines by W.H.O. Ethanolic and Aqueous extracts of the drug were used (40 μl/well) and the efficacy of the drug was compared with the standard drug i.e. Ciprofloxacin disk for Gram positive bacteria and Gentamicin for Gram negative strains and Control (Solvent used for dissolving the extracts i.e. Dimethyl Sulphoxide). Antibacterial effect was evaluated by measuring Zone of Inhibition (ZOI) (in mm). All the experiments were conducted in triplicates and in sterilized conditions. The results were analyzed statistically by using ANOVA. Phytochemical analysis of the drug confirmed the presence of alkaloids, flavonoids, glycosides, saponins, tannins and triterpenoids. Results indicate that *Caesalpinia bonducella* Roxb. has a significant activity against *Bacillus cereus*, *Coryne bacterium xerosis* and *Pseudomonas aeruginosa*, while moderate activity against *Proteus vulgaris* and *Staphylococcus aureus*. The study provides an *in-vitro* evidence of Karanjwa for having a very effective role against these pathogenic bacterial strains and can be considered as effective 'Drug Target' for further screening its effect in New Drug Development (NDD) in Research and Development (R & D) Unit in an effort to combat many infectious diseases.

Keywords: *Caesalpinia bonducella Roxb.*, CLSI Guidelines, Antibacterial effect, New Drug Development (NDL).

Introduction

Natural products have played a pivotal role in antibiotic drug discovery with most antibacterial drugs being derived from natural products (Buttler & Buss, 2006). The worldwide use of natural products including medicinal plants has become more and more important in primary health care especially in developing countries. With increased incidence of resistance to antibiotics, natural products from plants could be interesting alternatives. Some plant extracts and phytochemicals are known to have antimicrobial properties, and can be of great significance in therapeutic treatments. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century (Nair and Chanda, 2006).

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Caesalpinia bonducella Roxb. (Family : Caesalpinaceae) commonly known as 'Karanjwa' is used in Unani medicine since ancient times to treat various diseases. "Bonducella" the name of the species is derived from the Arabic word "Bonduce" meaning a "little ball" which indicates the globular shape of the seed *C. bonducella*. It is a climbing prickly shrub (Anonymous, 1987; Farooq, 2005) that grows in forests and near villages throughout the hotter parts of India. It has been used as an antipyretic, antimalarial, antimicrobial in traditional systems of medicine particularly in Unani Medicine (Ibne Sina, 1931; Hakim, 1343 H). Seeds of karanjwa are claimed to be styptic, purgative and anthelmintic and cures inflammations; useful in colic, malaria, hydrocele, skin diseases and leprosy (Anonymous, 1987; Chopra, 1958; Kiritikar and Basu, 1975). The powdered seeds mixed with equal part of pepper powder given to malaria patients were found to posses feeble antiperiodic properties (Farooq, 2005; Patil and Patil, 2007).

Seed of 'Karanjwa' and long pepper powders taken with honey gives good expectorant effect, The kernel of the seed is very useful and valuable in all cases of simple, continued and intermittent fevers (Ghani, 1921). Decoction of roasted kernels has been used in asthma (Chopra, 1958). Children unable to digest mother's milk are given the extract of the kernel or its powder along with ginger, salt and honey to get good stomachic effect (Farooq, 2005). Paste prepared from kernel gives relief from boils and other such swellings (Anonymous, 1950; Farooq, 2005). Chemically the seeds are found to contain a bitter substance bonducin, bonducellin phytosterinin, saponins (Rastogi and Mehrotra, 1993).

Present Work

The literature review undertaken on the test drug 'Karanjwa' reveals that the drug has been traditionally used by Unani physicians to combat many infectious diseases. Hence, the present study was designed to scientifically evaluate its antibacterial activity.

Material and Methods

Plant material: The herb was procured from the local market of Aligarh city and was properly identified by the available botanical literature (Fig. 1).

Preparation of plant extracts

The test drug was dried at room temperature in a ventilated room, milled to a fine powder and stored in a closed container in dark until use. Extraction was done according to the method described by Afaq *et al*. (1994) and Peach and Tracey (1955) with some minor modifications to maintain the low grade

temperature, keeping in mind that the thermo labile elements present in the drugs are destroyed when exposed to a higher temperature beyond 55^0C, so the heat wherever was needed was kept as low as possible to prevent the loss of thermo-labile substances present in the drugs from destruction. Strict aseptic precautions were followed throughout the process.

Aqueous extract

The coarse powdered drug was extracted using soxhlet apparatus, by reflux method with double distilled water (DDW) as a solvent at 50^0C for 6 hours or until the extracting return in the siphon was colorless. The extract obtained, was subjected to dryness in the Lyophilizer (Macro Scientific works, New Delhi) under reduced pressure.

Ethanolic extract: The coarse powdered drug was extracted with 95% ethanol as a solvent at 500C for 6 hours as above and dried under reduced pressure in the Lyophilizer. The stock solutions for aqueous and ethanolic extract was prepared from the dried extract so obtained in the DMSO as a solvent for use. The respective stock solutions so prepared were refrigerated till further use.

Phytochemical analysis

Phytochemical studies of the plant preparations are necessary for standardization, which helps in understanding the significance of phytoconstituents in terms of their observed activities. Phytochemistry also helps in standardizing the herbal preparations so as to get the optimal concentrations of known active constituents, and in preserving their activities. The qualitative phytochemical analysis of the drugs was done according to the scheme proposed by Bhattacharjee & Das (1969) and Afaq *et al*. (1994) and are presented in table-1.

Test microorganisms

Bacterial strains were selected on the basis of their clinical importance in causing diseases in humans. These were obtained from different sources, clinical isolates of *Staphylococcus aureus* , *Streptococcus mutans*, *Acetobacter bovis*, *Staphylococcus epidermidis*, *Bacillus cereus* and *Corynebacterium xerosis* were collected locally from Jawaharlal Nehru Medical College & Hospital; Interdisciplinary Biotechnology Unit; Microbiology Unit, Gandhi Eye Institute, Aligarh Muslim University, Aligarh, while standard strains were obtained from Hi-media Labs Pvt. Ltd., Mumbai, India and Microbial Type Culture Collection, Chandigarh, Punjab, India. The strains so selected for the study are *Staphylococcus aureus* (ATCC 29213), *Streptococcus mutans* (ATCC 25175),

Streptococcus pyrogenes (MTCC 435), *Staphylococcus epidermidis* (MTCC 435), *Bacillus cereus* (MTCC 430) and *Corynebacterium xerosis* (ATCC 373). All strains were incubated at 37^0C for 24 hours followed by frequent sub culturing to fresh media and were used as 'test bacteria', cultures were checked to confirm the presence of sufficient number of bacterial cells on nutrient broth and maintained on nutrient agar slant.

Medium

The solid media namely Nutrient Agar No.2 (NA) (M 1269S-500G, Hi-media Labs Pvt. Ltd, Bombay, India) was used for preparing nutrient plates, while Nutrient Broth (NB) (M002500G, Hi-media Labs Pvt. Ltd, Bombay, India) was used for the liquid culture media.

Antimicrobial Susceptibility Testing

Antibacterial tests were performed as CLSI Guidelines (Anonymous, 2003; Barry, 1999). Aqueous and ethanolic extract (40µl) in the concentration of 5 mg/ml dissolved in DMSO was used for its antimicrobial activity using Agar well diffusion (Ananthanarayan and Paniker, 2009) on solid media. Brain Heart Infusion (BHI) Agar (SM 211 Himedia Labs, Mumbai, India) was used for *S.mutans* while Mueller Hinton Agar No.2 (M1084 Hi media Labs, India) & Nutrient Agar (Himedia Labs, Mumbai, India) for preparing plates for rest of the bacterial strains. The solid Agar was punched with 6mm diameter wells. The inoculums ($1.5´10^8$ cfu/ml) were spread on to their respective agar plates using sterile swabs (PW041 Himedia



Fig. 1: Karanjwa seeds (*Caeasalpinia bonducella)*

Labs, Mumbai, India) and then filled with 40 ml extract. All the plates were incubated at 37^0C for 24 hours. Ciprofloxacin disk (SD-142, Himedia labs, Mumbai, India) was used as standard drug for Gram positive while Gentamicin (SD170 Hi media Labs, Mumbai, India) for Gram negative bacteria. Wells containing respective solvent (DMSO) served as control.

Growth Inhibition was recorded by measuring the diameter of the Inhibitory Zones after the period of incubation. Triplicates were maintained and the experiment was repeated thrice and the Mean values along with Standard error (Mean +S.E) are presented in Table-2 & 3 and comparison can be readily evaluated by Fig. 2 & 3.

Results and Discussion

The world is heavily populated with bacteria, viruses and fungi. Infections are the major cause of human diseases. Bacterial world itself is heavily populated

Table 1: Qualitative Analysis of the Phytochemicals in 'Karanjwa'

S.No.	Chemical Constituents	Test Reagents	Inference
1.	Alkaloids	Dragendorff reagent	+
		Wagner's reagent	+
		Mayer's reagent	+
2.	Carbohydrates	Molish Test	+
	Fehling Test	+	
		Benedict Test	+
3.	Flavonoids	Mg Ribbon and dil. Hcl	_
4.	Glycosides	NaOH Test	+
5.	Tannins/Phenols	Ferric Chloride Test	_
		Liebermann's test	_
		Lead Acetate test	+
6.	Proteins	Xanthoproteic test	_
		Biuret test	+
7.	Starch	Iodine Test	_
8.	Saponins	Frothing with $NaHCO_3$	_
9.	Steroids/Terpenes	Salkowski Reaction	+
10.	Resins	Acetic anhydride test	_

Indications: ' 'Absence and '+' Presence of constituents

Table 2: Antibacterial screening against Gram positive bacterial strains of Karanjwa (*Caesalpinia bonducella*)

S. No.	Strains	***Caesalpinia bonducella***		**Standard**
		Aqueous extract	**Ethanolic extract**	**Cipro-floxacin (30µgm)**
1.	*Staphylococcus aureus*	12±0.31*	12.4±0.40*	22*
2.	*Streptococcus mutans*	28.6±0.24*	11.33± 0.33*	21*
3.	*Streptococcus epidermidis*	_	_	23*
4.	*Staphylococcus pyrogenes*	_	_	22*
5.	*Corynebacterium xerosis*	26.6±0.50*	13.33±0.33*	21*
6.	*Bacillus cereus*	12.4±0.40*	12±0.31*	23*

*p-value>0.001

Table 3: Antibacterial screening against Gram negative bacterial strains of Karanjwa (*Caesalpinia bonducella*)

S. No.	Strains	***Caesalpinia bonducella***		**Standard**
		Aqueous extract	**Ethanolic extract**	**Gentamicin (30µgm)**
1.	*Escherichia coli*	11.33 ± 0.33*	17.2±0.37*	15*
2.	*Pseudomonas aeruginosa*	13.33 ± 0.33*	16±0.31*	14*
3.	*Proteus vulgaris*	_	_	14*
4.	*Klebsiella pneuomoniae*	11.33 ± 0.33*	19.2± 0.37*	15*

*p-value > 0.001

with too many species and produce fulminating infections like tetanus, gangrene, syphilis, gonorrhea, diphtheria, leprosy, tuberculosis, urinary tract infections, respiratory tract infections etc. (Mishal and Somani, 2000) in human beings and animals. The major problem that exists with the infectious diseases and the antibiotics used for them is the emergence of antibiotic resistance. This is a worldwide problem and now the time has come when one should think about its solution from alternative therapy. Evidence based medicine is the goal for western doctors nowadays and authorities request that for any drugs used in these medicines, they should solidify their evidence on the basis of scientific background to be presented, to make their use acceptable. So, natural drugs

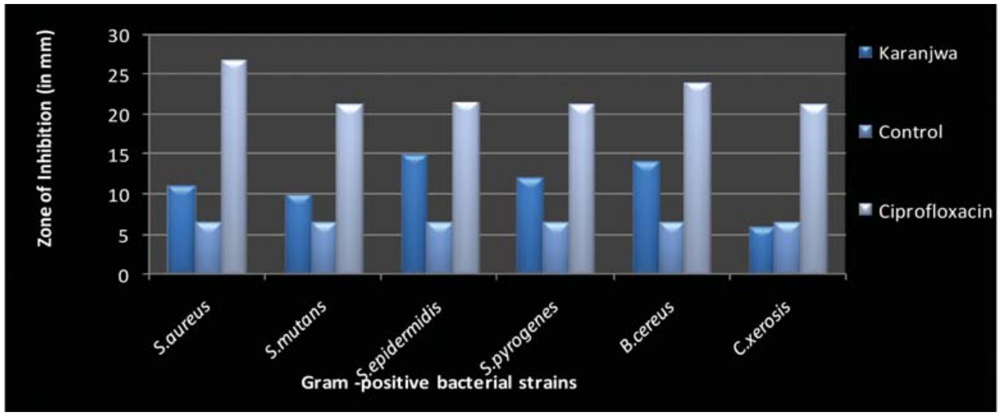


Figure 2: Antibacterial activity of Karanjwa extracts against Gram positive strains

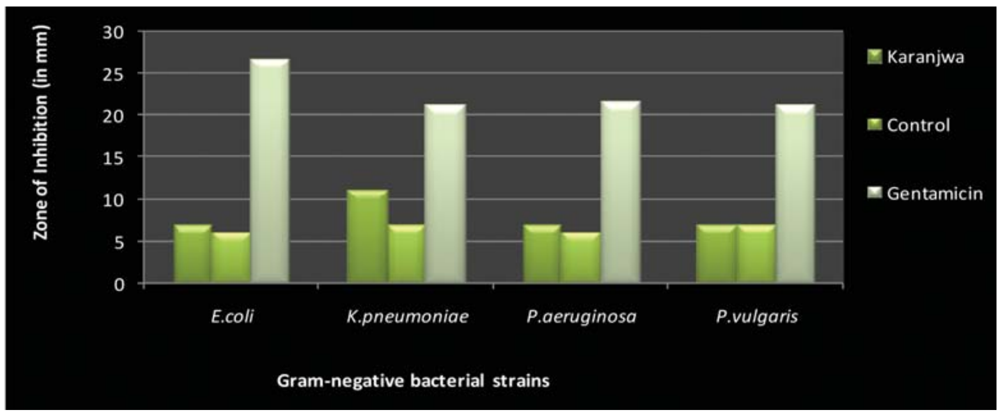


Figure 3: Antibacterial activity of Karanjwa extracts against Gram negative strains

fulfill this promise to a much extent as evident by the researches done so far. Certain antibiotics are there which have been derived from plant sources (Bhattacharjee and De, 2005). Keeping in mind, the side effects of antibiotics and emerging trends of development of resistance in microbes, present study assumes much significance in view of the fact that Karanjwa (*Caesalpinia bonducella* Roxb.) has been used in Unani medicine since ancient times for the treatment of infectious diseases, having great potential to kill or inhibit the growth of micro organisms.

Phytochemical analysis of *C. bonducella* has revealed the presence of alkaloids, flavonoids, glycosides, saponins, tannins and triterpenoids (Table-1). The results of antimicrobial activity of extracts showed a wide range of antibacterial activity against Gram positive and Gram negative bacteria. Results are expressed as ZOI (in mm) ± SEM (Standard error of mean). It was seen that aqueous extract of Karanjwa produces a significant results against *S.mutans, C.xerosis*, while the moderate activity was observe towards *S.aureus* and *B.cereus.* The ethanolic extract produces a significant ZOI against *E.coli*, *K.pneuomoniae*, *P.aeruginosa*,

moderate activity towards *S.aureus* and *B.cereus* and no activity was shown by either extract towards *S.pyrogenes, S.epidermidis* and *P.vulgaris*. Chemically the seeds are found to contain a bitter substance bonducin, bonducelli, phytosterinin, saponins (Rastogi and Mehrotra, 1993). These bitter component may be responsible for the antibacteria activity, however, further research work is needed to ensure its use clinically.

Conclusion

The study concludes that Karanjwa (*Caesalpinia bonducella* Roxb.) has a potent antibacterial activity against clinical and standards strains and thus could be used to derive antimicrobial agents to fight against the number of infectious diseases mainly against *S.mutans*, *B.cereus*, *C.xerosis, S.aureus, E.coli*, *K.pneuomoniae and P.aeruginosa.* This study not only provides the scientific evidence of antibacterial effect of 'Karanjwa' but also gives us a direction to search on, in detail, considering it as a Drug Target Molecule in New Drug Development series in an effort to discover new herbal antibiotics. The alarming and increasing scope of antibiotic resistance now further demands to search on for the newer drugs and establishing their antibacterial effect.

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Standardization of *Laooq-E-Khiyarshambar*: A Classical Unani Formulation

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Abstract

Standardization of Unani herbal formulations is very much essential to justify the quality of a medicine. Unani medicines have played a significant role in maintaining of human health. These medicines are accepted as important therapeutic agents for the treatment of various kinds of diseases. But in many instances, it has been noticed that incorrect raw materials have been added in the formulations which has resulted adulterated product in market place. The Unani medicine Laooq-e-Khiyarshambar is an important polyherbal Unani medicine is being used in the ailments of catarrh, coryza, bronchitis and constipation. The drug Laooq-e-Khiyarshamber was standardized using standard methods such as pharmacognostical, physico-chemical and TLC/HPTLC. The other parameters like microbial load, heavy metals, aflatoxins and pesticide residues were also analyzed to ascertain the quality of medicine. The physico-chemical data such as moisture content was 19.23%. Alcohol soluble extractives 22.68% and water soluble extractive 69.61% shows presence of polar compound and inorganic material respectively. The content of total ash was 1.50% and acid insoluble ash 0.07% shows negligible amount of siliceous matter present in the drug. HPTLC finger print of chloroform and alcohol extracts shows13and 9 peaks with the developing systems toluene: ethyl acetate 8.5: 1.5and 6:4 respectively. The data evolved can be adopted for laying down the pharmacopoeial standards and TLC/HPTLC finger prints of the drug Laooq-e-Khiyarshamber.

Keywords: Laooq-e-Khiyarshambar, Powder microscopy, Physico-chemical, TLC/HPTLC and, WHO parameters

Introduction

Laooq-e-Khiyarshambar (Anonymous, 2006) is one of the classical Unani formulation commonly used in Unani System of Medicine for different kind of ailments. This classical poly-herbal formulation is prepared using 5 ingredients (Table–1). The Physicians of Unani System of Medicine prescribes this drug for the treatment of Nazla (Catarrh), Zukam (Coryza), Sual (Bronchitis) and Qabz (Constipation) disorders. Standardisation of Unani medicines is very much essential to provide safe, efficacious and quality product for the needy mass. Due to lack of scientific standards and standard protocol there are batch to batch variations in the same finished compound formulation. The main requirement of a standardisation is to establish the presence of each ingredient in the formulations (Bandaranayake WM, 2006; Myers SP and Cheras PA, 2004). The present study was aimed to evaluate the drug using modern parameters like

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Table 1: Ingredients Composition of Laooq-e-Khiyarshambar

S.No.	Unani Name	Botanical Names	Part Used	Quantity
1.	Sapistan API-VI	*Cordia dichotama* Forst.f.	Fruit	187.5g
2.	Asl-us-Soos UPI-I	*Glycyrrhiza glabra* Linn.	Dried root and Stolon	187.5g
3.	Maghz-e-Floos-e-Khiyarshambar UPI-I	*Cassia fistula* Linn	Fruit pulp	250g
4.	Kateera UPI-V I	*Cochlospermum religiosum* Linn.	Gum	125g
5.	Qand safaid	Sugar	—	3.5Kg

microscopical, physico-chemical, thin layer chromatography and WHO parameters viz., microbial load, aflatoxin, heavy metals and pesticide residue.

Material and Methods

To standardize the drug Laooq-e-Khiyarshambar a systematic scheme of standardization was followed.

Collection of the raw drugs

The raw drugs namely Sapistan, Asl-us-Soos, Maghz-e-Floos-e-Khiyarshambar, Kateera and Qand safaid used in the preparation of drug were procured from raw drug dealers of Chennai market. The raw drugs were identified using pharmacognostical methods and developed their pharmacopoeial standards.

Preparation of the drug

As per the ingredients composition and guidelines of NFUM, Part – I, this classical ploy- herbal drug Laooq-e-Khiyarshambar was prepared in different batches at Laboratory scale.

Powder microscopy

Microscopical examination reveals more information of a drug and helps to identify the organised drugs by their well known histological characters viz., cell walls, cell contents, starch grains, calcium oxalate crystals, trichomes, fibres and vessels (Kokate *et al*., 2000).

The drug sample (5g) was weighed and mixed with 50ml of water in a beaker with gentle warming, till the sample completely dispersed in water. The mixture

was centrifuged and decanted the supernatant. The sediment was washed several times with distilled water, centrifuged again and decanted the supernatant. A few mg of the sediment was taken and mounted in glycerine. From this a few mg was taken in watch glass and added few drops of phloroglucinol and concentrated hydrochloric acid and mounted in glycerine. The microscopic salient features of the drug were observed in different mounts (Wallis, 1997; Johansen, 1940).

Physico-chemical analysis

To standardise the drug physico-chemical methods viz., moisture content, ash values, solubility in different solvents, pH values, bulk density and sugar content etc., were used. The drug samples were subjected for the evaluation of physico-chemical and quality control parameters and analysed as per the standards method (Anonymous, 1987).

Thin layer chromatography

In Thin Layer Chromatographic method two phases were used for separation of phytoconstitues; one of these is a stationary phase bed and other is a mobile phase which percolates through this bed. TLC is the best method for recording the finger prints which can be reproduced anywhere at the same laboratory condition of a particular product.

The samples of the drug (2g) were soaked in chloroform and alcohol separately for 18 hours, refluxed for ten minutes on water bath and filtered. The filtrates were concentrated on water bath and made up to 5ml in a standard flask separately and carried out the TLC studies (Wagner *et al.*, 1984).

Quality control parameters

To justify the quality and higher safety margins, the WHO has taken necessary steps. In order to ensure the quality of a drug the modern suitable techniques and standard methods were adopted. The parameters such as microbial load and heavy metal were carried out as per the WHO guidelines (Anonymous, 1998). Aflatoxin and pesticide residues were carried out by standard methods (Anonymous, 2000).

Results and Discussion

Organoleptic character

The drug Laooq-e-Khiyarshambar is a blackish brown semi-solid formulation with sweetish bitter in taste.

Microscopical observation

The powder microscopic study of the drug Laooq-e-Khiyarshambar was evaluated and observed the salient features of the raw drugs used in the preparation and the photographs are shown in Fig. – 1.

Stone cells (Sclereids) lignified, thick walled upto 250µ with broad and narrow lumen, cotyledonary parenchyma cells in surface view filled with aleurone grains and oil globules (Sapistan); fragments of reticulate and pitted vessels, reticulate vessels upto 150µ, crystal sheath of parenchyma cells containing a prism of calcium oxalate crystals upto 25µ, cork cells in surface view, parenchyma cells filed with starch grins, starch grains simple spherical upto 25µ (Asl-us-Soos).

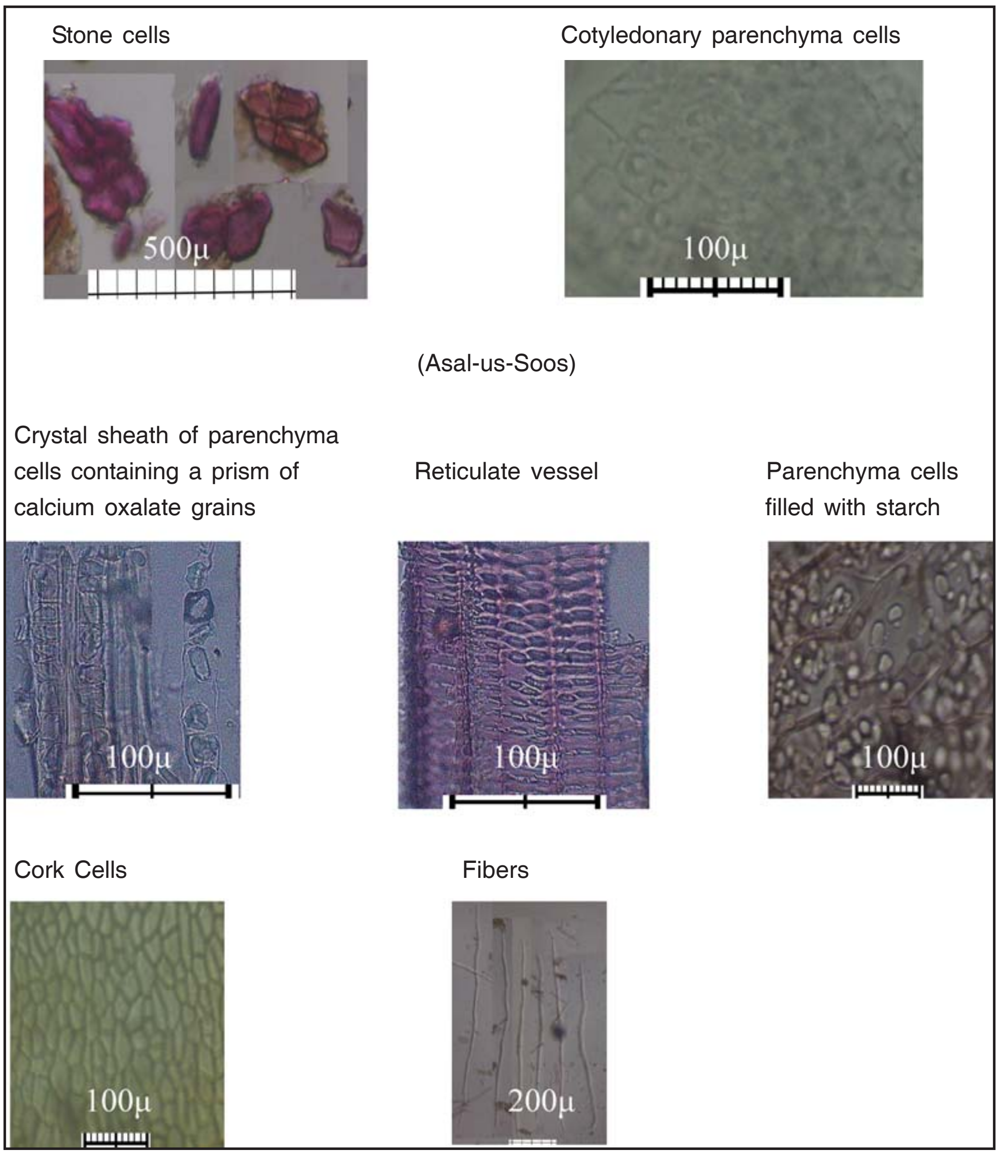


Fig. 1: Powder Microscopy (Sapistan)

Chemical analysis

The moisture content was obtained in the drug 19.23%. The physico-chemical data viz., alcohol soluble extractive (22.68%) might be due to the extraction of polar chemicals constituents and the water soluble extractives (69.61%) indicate the presence of inorganic constituents. The obtained data are shown in Table-2.

Thin Layer Chromatography Analysis

The TLC studies of chloroform and alcohol extract of three batch samples were carried out and observed. All the three samples showed identical spots at UV – 254nm, 366nm and in VS Reagent (Fig. I, II & III). The R_f values of chloroform extracts shows major spots at 0.72, 0.63, 0.60, 0.48, 0.35, 0.24 and 0.10 (Green) in UV-254. Under UV (366nm), it shows major spots at R_f 0.95 (Pink), 0.86 (Blue), 0.78 (Light blue), 0.71, 0.64 (Blue), 0.57 (Fluorescent blue), 0.53 (Blue), 0.45, 0.41, 0.31 and 0.15 (Fluorescent blue). Then dipped the plate in vanillin-sulphuric acid reagent followed by heating at 110° about 5 min and observed under visible light, the plate shows major spots at R_f 0.87 (Grey), 0.72, 0.69, 0.61, 0.46 (Pink), 0.36, 0.22 (Violet) and 0.11 (Green).

Then applied the alcohol extracts on TLC plate and developed the plate using Toluene: Ethyl acetate (6: 4) as mobile phase. After development allowed the plate to dry in air and examine under UV (254nm), it shows major spots at R_f

Table 2: Physico-chemical parameters

Parameters Analyzed	**Batch Number (n=3)**		
	I	**II**	**III**
Extractives			
Alcohol soluble matter	22.73%	22.44%	22.88%
Water soluble matter	69.76%	69.44%	69.64%
Ash			
Total ash	1.39%	1.59%	1.54%
Acid insoluble ash	0.09%	0.06%	0.07%
pH values			
1% Aqueous solution	5.17	5.21	5.11
10% Aqueous solution	4.47	4.54	4.41
Sugar estimation			
Reducing sugar	17.25%	17.19%	17.31%
Non-reducing sugar	3.11%	3.05%	3.40%
Moisture	19.18%	19.31%	19.20%
Bulk Density	1.4705	1.4609	1.4809

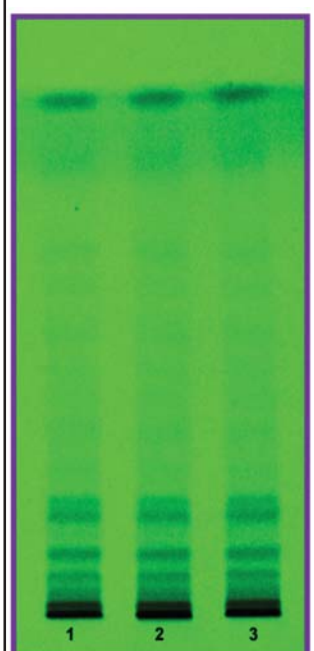
0.85, 0.68, 0.62, 0.53, 0.41, 0.34, 0.22, 0.18 and 0.12 (Green). Under UV (366nm), it shows major spots at R_f 0.96 (Pink), 0.86 (Fluorescent blue), 0.78, 0.70 (Blue), 0.62 (Fluorescent blue), 0.56 (Green), 0.52 (Blue), 0.45, 0.40, 0.30, 0.22 and 0.15 (Fluorescent blue). Dipped the plate in vanillin-sulphuric acid reagent followed by heating at 110° about 5 min and observed under visible light, the plate shows major spots at R_f 0.87 (Yellowish green), 0.72 (Light pink), 0.68, 0.62 (Pink), 0.45, 0.36 (Violet), 0.29 (Blue), 0.21 (Pink) and 0.15 (Green) (Fig.VI, VII & VIII).

Quality control parameters

The analyzed quality control parameters like microbial load and heavy metals were found within the permissible limit in the drug shown in Table - 3 and 4. The other parameters like aflatoxins B_1, B_2, G_1 and G_2 and pesticide residues - organo chlorine group, organo phosphorus group, acephate, chlordane, dimethoate, endosulphan, endosulfan, endosulfon, ethion, endosufon sulphate, fenthion, heptachlor, lindane, methoxychlor, phorate sulfoxide and phorate sulfone were not detected from the drug samples shown in Table - 5 and 6.

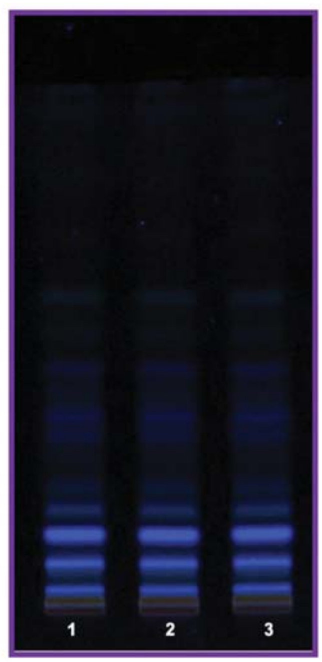
Thin Layer Chromatography
(Chloroform Extract)

Fig.I



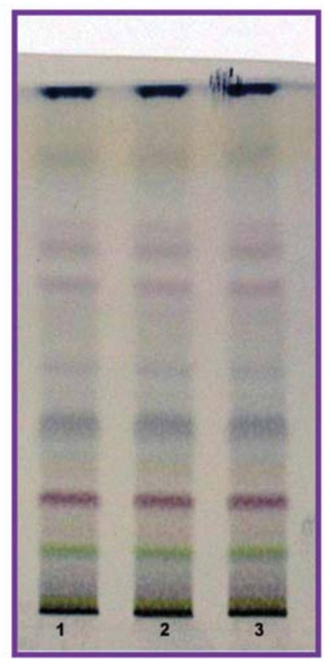
UV - 254 nm

Fig.II



UV - 366 nm

Fig.III

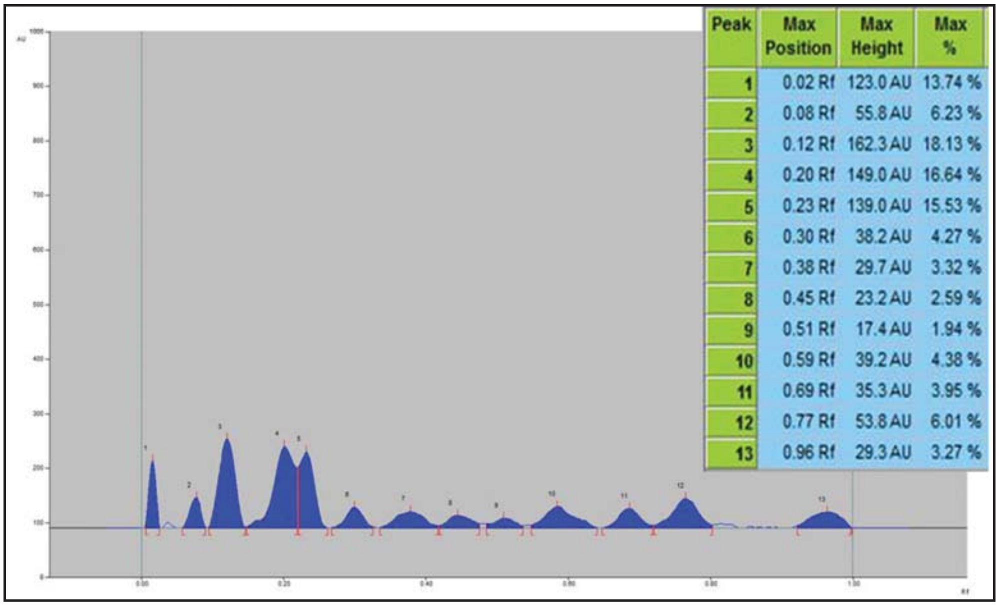


V - S Reagent

Solvent System : Toluene :Ethyl acetate (8.5:1.5)
Track 1. Batch - I; Track 2. Batch - II; Track 3. Batch - III

HPTLC finger print of chloroform extract

TLC plate was developed using Toluene: Ethyl acetate (8.5: 1.5) as mobile phase. After development allow the plate to dry in air, record the finger print (Fig. IV) and densitometric chromatogram (Fig .V) of the three batch samples of the compound formulation at 254 nm.



Peak	Max Position	Max Height	Max %
1	0.02 Rf	123.0 AU	13.74 %
2	0.08 Rf	55.8 AU	6.23 %
3	0.12 Rf	162.3 AU	18.13 %
4	0.20 Rf	149.0 AU	16.64 %
5	0.23 Rf	139.0 AU	15.53 %
6	0.30 Rf	38.2 AU	4.27 %
7	0.38 Rf	29.7 AU	3.32 %
8	0.45 Rf	23.2 AU	2.59 %
9	0.51 Rf	17.4 AU	1.94 %
10	0.59 Rf	39.2 AU	4.38 %
11	0.69 Rf	35.3 AU	3.95 %
12	0.77 Rf	53.8 AU	6.01 %
13	0.96 Rf	29.3 AU	3.27 %

Fig. IV: HPTLC finger print of Laooq-e-Khiyarshambar chloroform extract at 254 nm

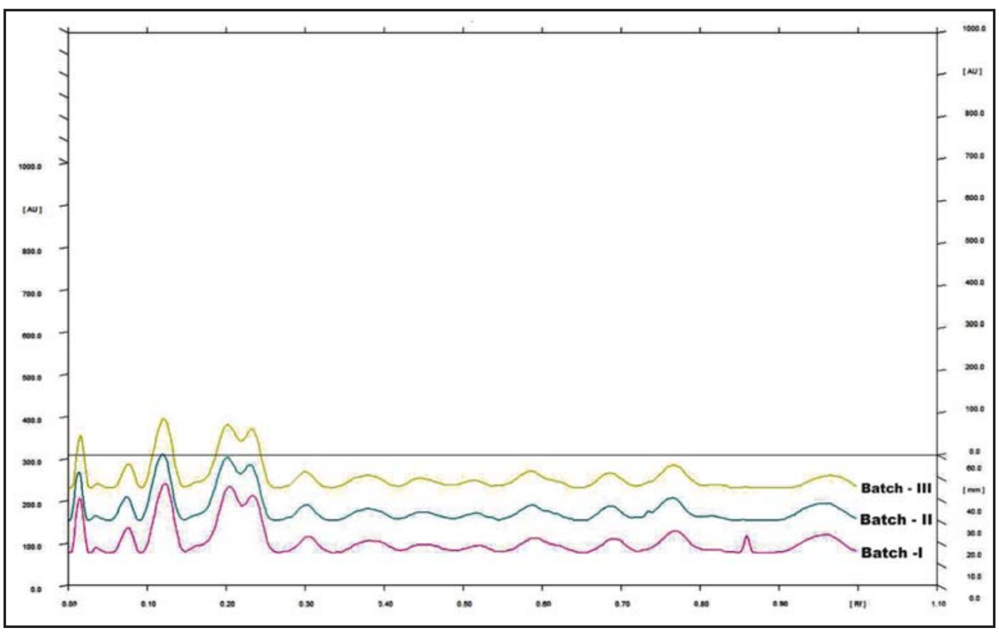


Fig. V: Densitometric chromatogram of Laooq-e-Khiyarshambar chloroform extracts at 254 nm

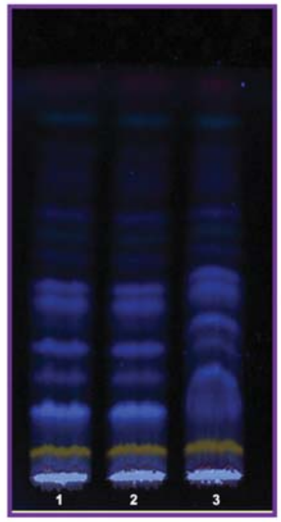
Thin Layer Chromatography
(Alcohol Extract)

Fig.VI



UV - 254 nm

Fig.VII



UV - 366 nm

Fig.VIII



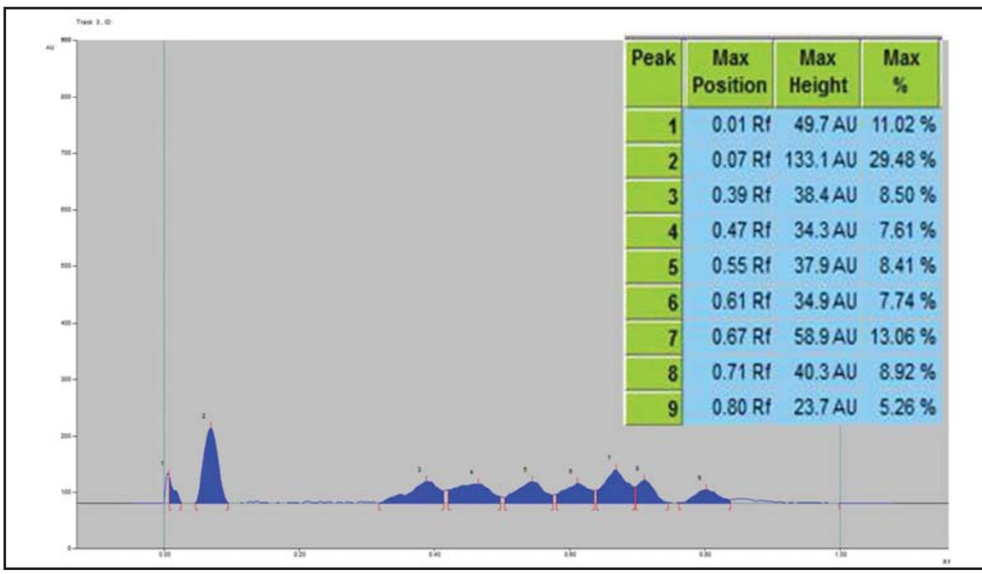
V - S Reagent

Solvent System : Toluene :Ethyl acetate (6:4)

Track 1. Batch - I; Track 2. Batch - II; Track 3. Batch - III

HPTLC finger print of alcohol extract

TLC plate was developed using Toluene: Ethyl acetate (6: 4) as mobile phase. After development allow the plate to dry in air, record the finger print (Fig .IX) and densitometric chromatogram (Fig. X) of the three batch samples of the compound formulation at 254 nm.



Peak	Max Position	Max Height	Max %
1	0.01 Rf	49.7 AU	11.02 %
2	0.07 Rf	133.1 AU	29.48 %
3	0.39 Rf	38.4 AU	8.50 %
4	0.47 Rf	34.3 AU	7.61 %
5	0.55 Rf	37.9 AU	8.41 %
6	0.61 Rf	34.9 AU	7.74 %
7	0.67 Rf	58.9 AU	13.06 %
8	0.71 Rf	40.3 AU	8.92 %
9	0.80 Rf	23.7 AU	5.26 %

Fig.IX: HPTLC finger print of Laooq-e-Khiyarshambar alcohol extract at 254 nm

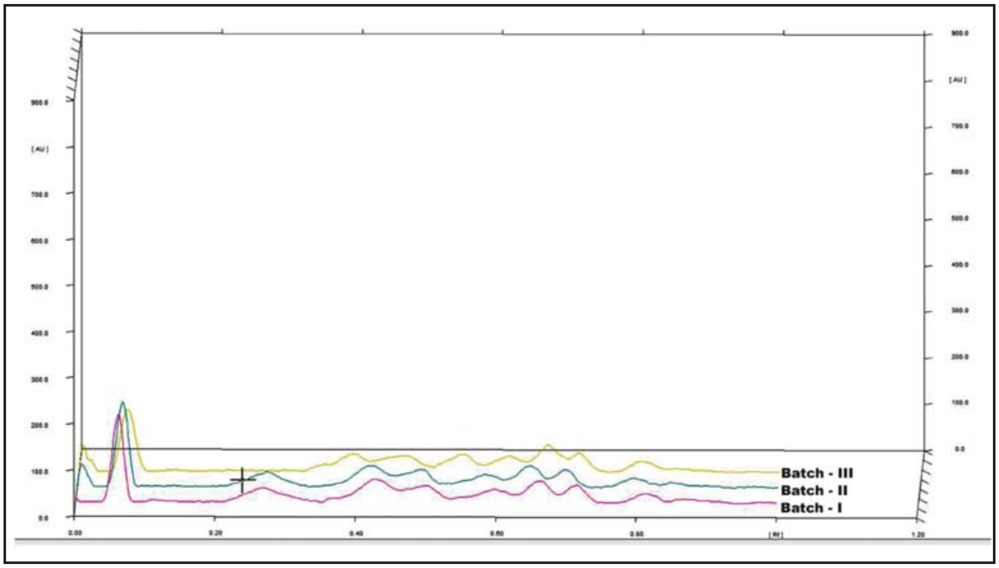


Fig. X: Densitometric chromatogram of Laooq-e-Khiyarshambar alcohol extracts at 254 nm

Table 3: Analysis of Microbial load

S.No.	Parameter Analyzed	Results	WHO Limits
1	Total Bacterial Count	7 x 10^3 CFU/gram	10^5 CFU / gm
2	Total Fungal Count	- Less than 10 CFU/gram	10^3 CFU / gm
3	Enterobacteriaceae	Absent / gm	10^3 CFU / gm
4	Salmonella	Absent / gm	Nil
5	*Staphylococcus aureus*	Absent / gm	Nil

Table 4: Estimation of Heavy Metals

S.No.	Parameter Analyzed	Results	WHO & FDA Limits
1	Arsenic	Not detected	3 ppm
2	Cadmium	Not detected	0.3 ppm
3	Lead	0.0011	10 ppm
4	Mercury	Not detected	1.0 ppm

Table 5: Estimation of Aflatoxins

S.No.	Aflatoxins	Results	WHO Limits
1	B_1	ND	0. 5ppb
2	B_2	ND	0.1ppb
3	G_1	ND	0. 5ppb
4	G_2	ND	0.1ppb

ND = Not Detected

Table 6: Analysis of Pesticide Residues

S.No.	Pesticide Residues	Results	Limits
1	Organo Chlorine group	ND	(DL 0.005mg/Kg)
2	Organo Phosphorus group	ND	(DL 0.005mg/Kg)
3	Acephate	ND	(DL 0.005mg/Kg)
4	Chlordane	ND	(DL 0.005mg/Kg)
5	Dimethoate	ND	(DL 0.005mg/Kg)
6	Endosulphan	ND	(DL 0.005mg/Kg)
7	Endosulfan	ND	(DL 0.005mg/Kg)
8	Endosulfon	ND	(DL 0.005mg/Kg)
9	Ethion	ND	(DL 0.005mg/Kg)
10	Endosufon sulphate	ND	(DL 0.005mg/Kg)
11	Fenthion	ND	(DL 0.005mg/Kg)
12	Heptachlor	ND	(DL 0.005mg/Kg)
13	Lindane	ND	(DL 0.005mg/Kg)
14	Methoxychlor	ND	(DL 0.005mg/Kg)
15	Phorate sulfoxide	ND	(DL 0.005mg/Kg)
16	Phorate sulfone	ND	(DL 0.005mg/Kg)

ND – Not detected

Conclusion

The evaluated data such as powder microscopy, physico-chemical, TLC/HPTLC fingerprints and analysis of quality control parameters indicates that the genuine raw drugs were added in the formulation and there is no variation in the batch to batch consistency of the drug.

Acknowledgement

The authors are extremely thankful to the Director General, CCRUM, New Delhi, for his valuable guidance, encouragement and providing necessary research facilities to carry out the present studies.

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Standardization of Amber-e-Ash-hab: A Premium Unani Marine Drug

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Abstract

The current study is an attempt to standardize one of the important Unani drugs of the animal origin Amber-e-Ash-hab (Ambergris). Amber is used in various Unani formulations as it has tremendous medicinal properties and sanative effects. In some parts of Asia & Africa it was used as flavouring for food. It was also widely used in perfumery industry as a perfumery fixative; Amber is the slowest of all perfume materials to evaporate. Though it has now been substantially displaced by synthetics in perfumery industry. Amber has been used in many traditional systems of medicines for ages but due to its high cost and low availability, it is sometimes replaced by or adulterated with low quality substances. In order to check the adulteration and to make its precise identification, the drug was tested through Physico-chemical parameters, TLC, U.V. Spectroscopy etc.

Keywords: Amber-e-Ash-hab, TLC, U.V. Spectroscopy.

Introduction

Amber-e-Ash-hab (Amber) or Ambergris got its name from French 'ambre gris' which means grey amber. In fact the European name Ambergris was derived from 'Anbar' which was given to it by Arabian society. Due to its restorative and aphrodisiac effects (Wittop, 1972; Taha, 1989), Amber is used in many Unani formulations to a great advantage in the treatment of Zof-e-Bah (sexual debility), Zof-e-Aam (general debility) and Zof-e-Aza-e-Raeesa (weakness of the principal organs like heart, brain, liver etc.) (Anonymous, 2006). Arq-e-Amber, Habb-e-Amber, Habb-e-Amber Momyaee, Dawa-ul-Misk Motadil Sada, Dawa-ul-Misk Motadil Jawahirwali are quite a few Unani formulations to mention where Amber is used as one of the ingredients for its excellent medicinal values (Anonymous, 2006).

Amber is a solid waxy substance produced as a biliary secretion of the intestines of the sperm whale. It is thought to be a substance protective against intestinal irritation caused by the indigestible beaks of squid and cuttlefish that the sperm whale feeds upon. The whale's intestine can hold only small lumps of amber. The larger pieces are regurgitated.

Amber can be found floating upon the sea or in the sand near the coast. It is usually found in the Atlantic Ocean; on the coasts of Africa, Brazil, Madagascar, The Maldives, China, Japan, India, Australia, and New Zealand (Parry, 1925). Most commercially collected Amber comes from the Bahamas Islands in the Caribbean.

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Amber is found in different shapes and sizes weighing up to 100kg (Burfield T., 2000). Fresh Amber is almost black and soft and has a disagreeable odour. After months of photo-degradation or oxidation in the ocean, it gradually hardens and develops a crusty & waxy texture, light grey colour and a pleasant smell (Wittop, 1972; Ohloff, 1980; Charles, 1990). The colour of Amber varies from black or dark brown to grey or yellow which tends to lighten with time. The chief constituent of Amber is ambrein which on auto-oxidation results in the formation of ambrinol and ambroxan, the main fragrant components of Amber (Karl-Georg Fahlbusch *et al.*, 2007).

Materials and Methods

Amber was procured from raw drug dealer, New Delhi. It was free from any foreign matter. The sample was crushed to a coarse powder with the help of mortar and pestle (Fig. 1).

Physico-chemical parameters

Physico-chemical parameters like foreign matter, solubility in ethanol, water and hexane, total ash, acid in-soluble ash were carried out as per standard methods (Anonymous, 1998).



Figure 1: Amber

Preparation of extract for TLC

0.5g of the drug sample was dissolved in 50ml of pet. ether (60^0-80^0) and refluxed for 20 minutes on a water bath. This extract was used as such for Thin Layer Chromatography (Wagner *et al.*, 1984; Stahl, 1996).

Preparation of extract for U.V. Spectroscopic studies

1g drug was dissolved in 100ml of pet. ether (60^0-80^0) and refluxed for 15 minutes on water bath. The solution was made up to 100ml in a volumetric flask. This solution was used as such for U. V. spectroscopic analysis and pure pet. ether (60^0-80^0) was used as a blank solution (Willard *et al.*, 1965).

Observations

Amber-e-Ash-hab was dark grey in colour with yellowish tint and had a waxy texture. It was free from any foreign matter. It melted at about 65^0 C to a fatty resinous liquid and at 100^0 C it vaporized into white fumes (Dauphin, ynm; Reis, 2013). These distinctive properties show that the sample of Amber was authentic.

Results and Discussion

Chemical Analysis

The physico-chemical data of the drug are shown in Table-1. The water soluble extractive (0.38 – 0.42%) indicates that Amber is almost insoluble in water due to its waxy nature; on the other hand hexane soluble extractive (71.22 – 72.10%) shows that it is highly soluble in liquid hydrocarbons. The low value of acid insoluble ash of the drug indicates that the drug is free from siliceous matter. The heavy metal contents are below detectable limits (Table 2).

Thin Layer Chromatography Analysis

TLC of pet. ether (60^0-80^0) extract of the drug was carried out on pre-coated plate of Silica Gel 60 F_{254} (E. Merck) using the solvent system of toluene – ethyl

Table 1: Physico-chemical Parameters

S.No.	Parameters	Values (%)
1.	Ethanol soluble extractive	6.75 – 7.02
2.	Water soluble extractive	0.38 – 0.42
3.	Hexane soluble extractive	71.22 – 72.10
4.	Total ash	0.54 – 0.60
5.	Acid insoluble ash	0.21 – 0.25

acetate (9:1). It showed six spots after spraying the plate with 2% ethanolic sulphuric acid followed by heating for about 10 minutes at 105^0 C in an oven (Table 3, Fig.2).

Table 2: Heavy Metals

S.No.	Parameters	Values	WHO Limits
1.	Arsenic	Not detected	10 ppm
2.	Cadmium	Not detected	03 ppm
3.	Lead	Not detected	10 ppm
4.	Mercury	Not detected	1.0 ppm

Table 3: TLC Results

Extract	Solvent System	Spraying reagent	No. of Spots	Rf Values with colour
Pet. ether (60^0-80^0)	Toluene – Ethyl acetate (9 : 1)	2% Ethanolic Sulphuric acid	06	0.18 (Pink) 0.30 (Pink) 0.33 (Pink) 0.42 (Purple) 0.49 (Brownish orange) 0.83 (Peach)



Figure 2: TLC of Amber

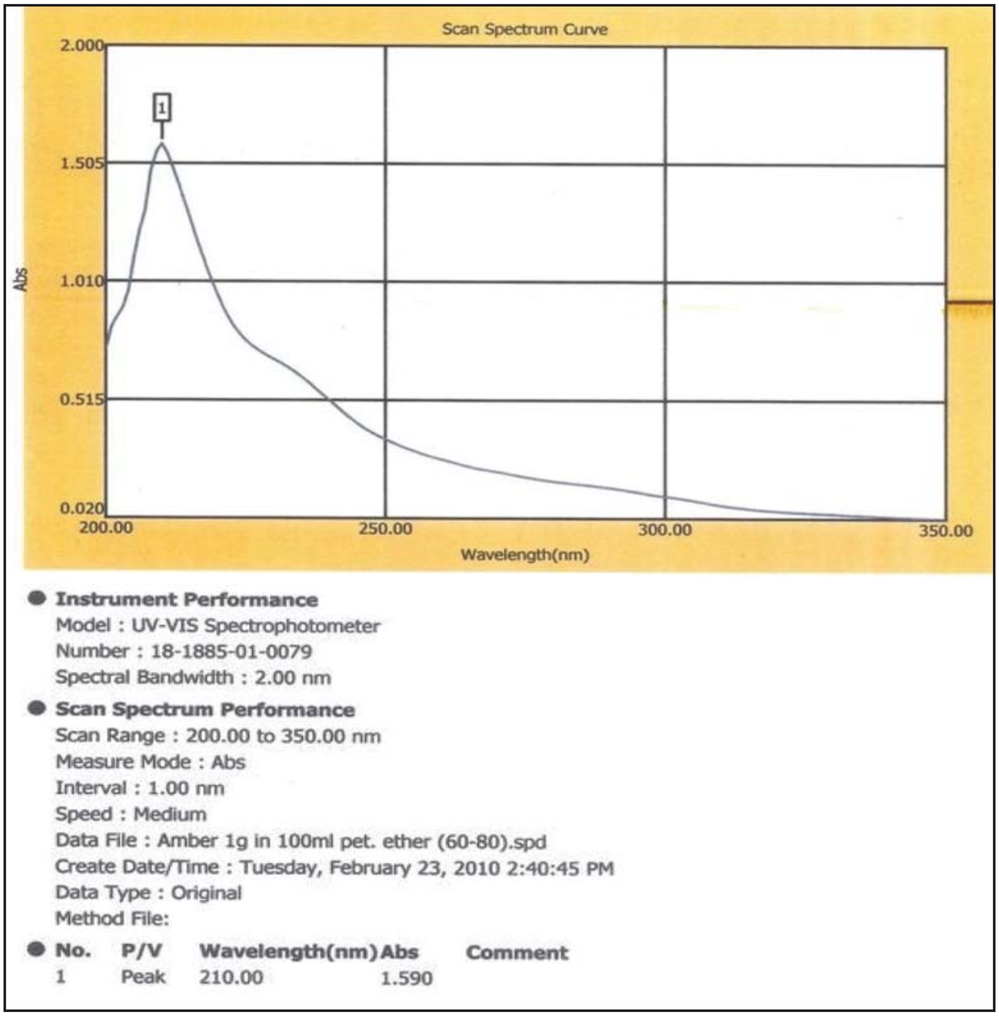


Figure 3: U. V. Spectrum of Amber-e-Ash-hab

U.V. Spectroscopic Studies

The U V spectrum of Amber shows a characteristic peak at 210nm due to an absorbance of 1.590 by the drug (Fig. 3). The sharp peak, without any noise, strengthens that the drug is pure.

Conclusion

It is difficult to ascertain the authenticity of Amber as it is found in various shapes and sizes. Its colour also varies to a great extent. The present study, therefore, holds high significance as various physico-chemical standards, TLC profile and U. V. spectrum provide criteria for easy identification of real Amber-e-Ash-hab and ensure the quality of the drug.

Acknowledgement

The authors are extremely thankful to the Director-General, CCRUM, New Delhi, for encouragement and providing necessary research facilities to carry out this work.

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Pharmacopoeial Standardization of Unani Formulation Majoone-e-Lana

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Abstract

Standardization of herbal formulation is essential in order to assess the quality, purity, safety, and efficacy of drugs based on the analysis of their active properties. Testing of herbal preparations using scientific methodologies will add to quality and authenticity of the product. This article reports standardization parameters for Unani formulation Majoon-e- Lana used traditionally in the treatment of Muqawwi-e-Asab (nerve strengthening), Zof-e-Asab (neurasthenia), Falij (hemiplegia), Laqwa (facial paralysis), Rasha (tremor, trembling), Waj-ul-Mafasil (arthralgia) and Sara (epilepsy). Majoon-e-Lana is one of the Unani poly herbal formulations was prepared with the combination of twenty one ingredients as per National Formulary of Unani Medicine, and it was standardized by organoleptic characterization, physicochemical testing, thin layer chromatography/high performance thin layer chromatography, microbial load, heavy metal analysis, aflatoxins and pesticidal residues profiling employing a standard methodology. The physico-chemical data and TLC/HPTLC finger print analysis evolved can be adopted for laying down the pharmacopoeial standards for Majoon-e-Lana. All three different batch samples were found to be safe when tested for the heavy metal contamination, microbial load, aflatoxins and pesticide residues. Results of the experiments conducted provided diagnostic characteristics to identify and standardize the formulation prepared using ingredients of Majoon-e-Lana.

Keywords: Majoon-e-Lana, Physico-chemical parameters, TLC/HPTLC finger print, WHO parameters.

Introduction

Many modern medicines are directly or indirectly derived from higher plants (WHO, 2005). All medicines, whether synthetic or of plant origin, should fulfill the basic requirements of being safe and effective (EMEA, 2005; WHO, 2002). Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, and definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. Quality of raw materials, good agricultural practices and good manufacturing practices play fundamental roles in guaranteeing the quality and stability of herbal preparations (WHO, 2000). Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular herbal medicine. Hence, standardization is a tool used in the quality control process (Kunle, 2012).

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Majoon-e-Lana is used in the ailments of Nerve strengthening, Neurasthenia, Hemiplegia, Facial paralysis, Tremor, Trembling, Arthralgia and Epilepsy. The present paper deals the physicochemical, TLC/HPTLC finger print, heavy metals, microbial load, aflatoxins and pesticide residues.

Materials and Methods

All the ingredients were procured from the local market and identified. Specimens of all ingredients of the formulation have been deposited in the museum of Drug Standardization Research Unit at Regional Research Institute of Unani Medicine, Chennai, Tamil Nadu, India. The drug Majoon-e-Lana was prepared as per the formulation composition given in NFUM, Part-I using 21 ingredients showed in table 1 (Anonymous, 1981).

Table 1: List of ingredients of Majoon-e-Lana

Azaraqi Mudabhar	Strychnos nux-vomica Linn.	Seed	20g
Filfil Safaid	*Piper nigrum* Linn.	Fruit	10g
Filfil Siyah	*Piper nigrum* Linn.	Fruit	10g
Darchini	*Cinnamomum zeylanicum* Blume.	Inner stem bark	10g
Filfil Daraz	*Piper longum* Linn.	Fruit	10g
Jauzbuwa	*Myristica fragrans* Houtt.	Endosperm	10g
Bisbasa	*Myristica fragrans* Houtt.	Arillus	10g
Mastagi	*Pistacia lentisus* Linn.	Resin	10g
Sad Kufi	*Cyperus rotundus* Linn.	Rhizome	10g
Zanjabeel	*Zingiber officianale* Rosc.	Rhizome	10g
Qaranful	*Syzygium aromaticum* Merr & L.M. Perry	Flower bud	10g
Aamla	*Emblica officinalis* Gaertn.	Fruit	10g
Sumul-ut-Teeb	*Nardostachys jatamansi* Dc.	Rhizome	10g
Heel Khurd	*Elettaria cardamomum* Maton.	Fruit	10g
Nankhwah	*Trachyspermum ammi* (L.) Sprague. ex Turril	Fruit	10g
Badiyan	*Foeniculum vulgare* Mill.	Fruit	10g
Zafran	*Crocus sativus* Linn.	Stamen & Stigma	10g
Sandal Safaid	*Santalum album* Linn.	Heart wood	10g
Ood-e-Balsan	*Commiphora opobalsamum* (L.) Engl.	Wood	10g
Agar	*Aquilaria agallocha* Roxb.	Heart wood	10g
Qand Safaid	Sugar	—	600g

Method of preparation of the drug

All the ingredients were taken of pharmacopoeial quality. Cleaned, dried, powdered and sieved through 80 mesh. Mixed the powders of all the ingredients of Azaraqi Mudabhar, Filfil Safaid, Filfil Siyah, Darchini, Filfil Daraz, Jauzbuwa, Bisbasa, Mastagi, Sad Kufi, Zanjabeel, Qaranful, Aamla, Sumul-ut-Teeb, Heel Khurd, Nankhwah, Badiyan, Zafran, Sandal Safaid, Ood-e-Balsan and Agar, kept separately. Dissolved the specified quantity of ingredient Qand Safaid on slow heat in 600 ml of water, at the boiling stage added 0.1% citric acid and mixed thoroughly. At the stage of 70% consistencies of quiwam, 0.1% sodium benzoate was added and mixed thoroughly to prepare the quiwam of 76% consistency. Removed the vessel from the fire, while hot condition the mixed powders of all the ingredients were added and mixed thoroughly to prepare the homogenous product. Allowed it to cool to room temperature and packed in tightly closed containers to protect from light and moisture.

Physico-chemical analysis

The analytical data like moisture content, ash values, alcohol and water soluble extractives, pH values, bulk density and estimation of sugar were arrived by employing the standard procedure (Anonymous, 1998 and Anonymous, 1987).

TLC/HPTLC finger print analysis

Preparation of extracts for TLC

The formulations of the three batch samples were extracted with chloroform and alcohol. The extracts were concentrated and made up to 10 ml in a volumetric flask separately. These solutions were used for the TLC/HPTLC finger print analysis.

The TLC/HPTLC finger print analysis of chloroform and alcohol extracts of the formulations were performed using aluminium plate precoated with silica gel 60 F_{254} (E.merck) employing CAMAG Linomat IV sample applicator. The chromatogram were developed using the developing systems toluene: ethyl acetate (9: 1) and toluene: ethyl acetate (6: 4) for chloroform and alcohol extracts respectively. The plates were dried at room temperature and observed the spots at UV-254 nm, UV-366 nm and the plates were scanned at 254 nm to record the finger print spectrum. Finally the plate were dipped in vanillin-sulphuric acid and heated at 105° C till coloured spots appeared (Wagner and Bladt, 1984; Sethi, 1996).

Estimation of microbial load

The estimation of microbial load viz. total bacterial count (TBC), total fungal count (TFC), Enterobacteriaceae, *Escherichia coli*, *Salmonella* spp and *Staphylococcus aureus* were determined as per WHO standards (1998).

Estimation of Heavy Metals

The procedure was used for the analysis of heavy metals like lead, cadmium, mercury and arsenic as per WHO, 1998 and AOAC, 2005.

Instrument details and operating parameters

Thermo Fisher M Series, 650902 V1.27 model Atomic Absorption Spectrometer (AAS) was used for the analysis. The operating parameters:

Lead and Cadmium: Instrument technique - Flame technique; wavelength (Lead) - 217 nm; wavelength (Cadmium) - 228.8 nm; slit width - 0. 5 mm; lamp current (Pb) - 4.0 mA; lamp current (Cd) - 3.0 mA; carrier gas and flow rate - air and acetylene, 1.1 L/min; sample flow rate - 2 ml/min. Mercury: Instrument technique - Cold vapour technique; wavelength - 253.7 nm; slit width - 0. 5 mm; lamp current - 3.0 mA; carrier gas and flow rate - argon, 1.1 L/min; sample flow rate - 5ml/min. Arsenic: Instrument technique - Flame vapour technique; wavelength - 193.7 nm; slit width - 0. 5 mm; lamp current - 6.0 mA; carrier gas and flow rate - acetylene, argon, 1.1 L/min; sample flow rate - 5ml/min. The Hallow cathode lamp for Pb, Cd, Hg and As analysis were used as light source to provide specific wavelength for the elements to be determined.

Analysis of Aflatoxins

The procedure was followed for the analysis of aflatoxins B_1, B_2, G_1 and G_2 as per Official Analytical Methods of the American Spice Trade Association (ASTA) (1997).

Instrument details and operating parameters

Thermo Fisher High Performance Liquid Chromatography (HPLC) was used for the aflatoxins analysis. Column - Ultra C18, 250 X 4.6 mm, 5 µm particles; mobile phase - water: acetonitrile: methanol (65: 22.5: 22.5); flow rate - 1 ml/min; temperature - 35º C; detector - fluorescence detector at 360 nm; injection - 20 µl (Aflatoxins mixture and sample)

Analysis of pesticide residue

The procedure was followed for the analysis of pesticidal residues as per AOAC, 2005. Pesticidal residues were analyzed by Gas Chromatography-Mass Spectra

(GC-MS)(Instrument-Agilent, detector-mass selective detector, column specification-DB5MS, carrier gas- helium, flow rate-1ml/min, column length- 30 m, internal diameter-0.25 mm, column thickness-0.25 μm).

Results and Discussion

The drug is brown in colour, semi-solid, characteristic of its own odour and sweetish bitter in taste.

Physico-chemical parameters

Physico-chemical parameters of Majoon-e-Lana are tabulated in Table-2. Quantitative standards revealed that the moisture content was 19.65%, ash content was 1.37% and acid insoluble ash 0.46% indicates the negligible amount of siliceous matter present in the drug. The water soluble extractive value of the drug 66.36% indicates the presence of inorganic content and the alcohol soluble extractive value 34.53% indicates the extraction of polar constituents.

Table 2: Physico-chemical parameters of the Majoon-e-Lana

S.No.	Parameters	Majoon-e-Lana		
		Batch-I	Batch-II	Batch-III
1	Moisture (% w/w)	19.44	19.78	19.74
2	Extractive values (% w/w)			
	Alcohol soluble matter	34.56	34.75	34.28
	Water soluble matter	66.14	66.40	66.56
3	Ash values (% w/w)			
	Total ash	1.62	1.28	1.23
	Acid insoluble ash	0.60	0.43	0.37
4	*pH* values			
	1% Aqueous solution	5.59	5.42	5.27
	10% Aqueous solution	4.65	4.51	4.42
5	Sugar estimation			
	Reducing sugar (% w/w)	41.40	41.34	41.55
	Non reducing sugar (% w/w)	9.42	9.36	9.49
6	Bulk Density	1.6509	1.6501	1.6405

All values are mean of three determinations

TLC studies of chloroform extract

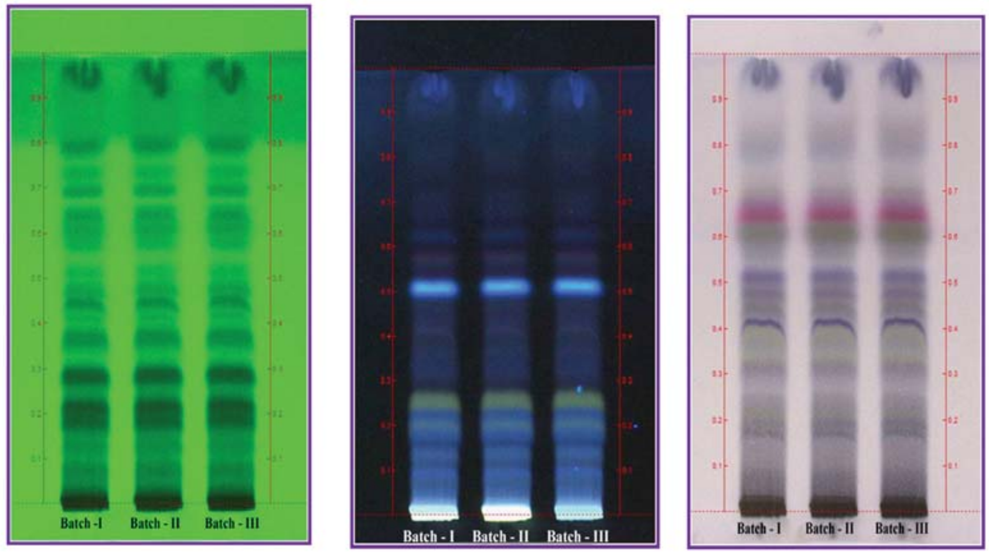
The TLC studies of chloroform extract are tabulated in Table - 3. All the three batch samples showed identical spots in UV-254 nm, UV-366 nm and visible light (after derivatised with vanillin – sulphuric acid reagent). In UV – 254 nm, 366 nm and visible light it shows 15, 10 and 12 spots respectively with different R_f values (Fig. 1).

HPTLC finger print studies of chloroform extract

The finger print of the chloroform extract shows 15 peaks of which peaks at R_f 0.24, 0.32, 0.42, 0.52, 0.70, 0.73, 0.80 and 0.92 were the major peak whereas peaks at R_f 0.02, 0.07, 0.14, 0.47, 0.54 and 0.85 were moderately smaller peaks (Fig.2). The HPTLC densitometry chromatogram of chloroform extract of three batch samples were found to be same when scanned at 254 nm (Fig. 3).

Table 3: R_f values of the chloroform extract

Solvent System	***Rf* Values**		
	UV- 254 nm	**UV – 366 nm**	**Visible light (after derivatization with vanillin – sulphuric acid reagent)**
Toluene: Ethyl acetate (9:1)	0.79 Green	0.80 Light blue	0.84 Brown
	0.74 Green	0.68 Light blue	0.79 Violet
	0.70 Green	0.63 Blue	0.70 Grey
	0.65 Green	0.57 Blue	0.65 Red
	0.61 Green	0.51 Fluorescent blue	0.61 Grey
	0.58 Green	0.41 Blue	0.52 Violet
	0.52 Green	0.26 Yellowish green	0.48 Violet
	0.48 Green	0.22 Violet	0.45 Grey
	0.45 Green	0.18 Blue	0.42 Violet
	0.41 Green	0.14 Blue	0.32 Grey
	0.37 Green		0.24 Grey
	0.29 Green		0.19 Violet
	0.22 Green		
	0.18 Green		
	0.13 Green		

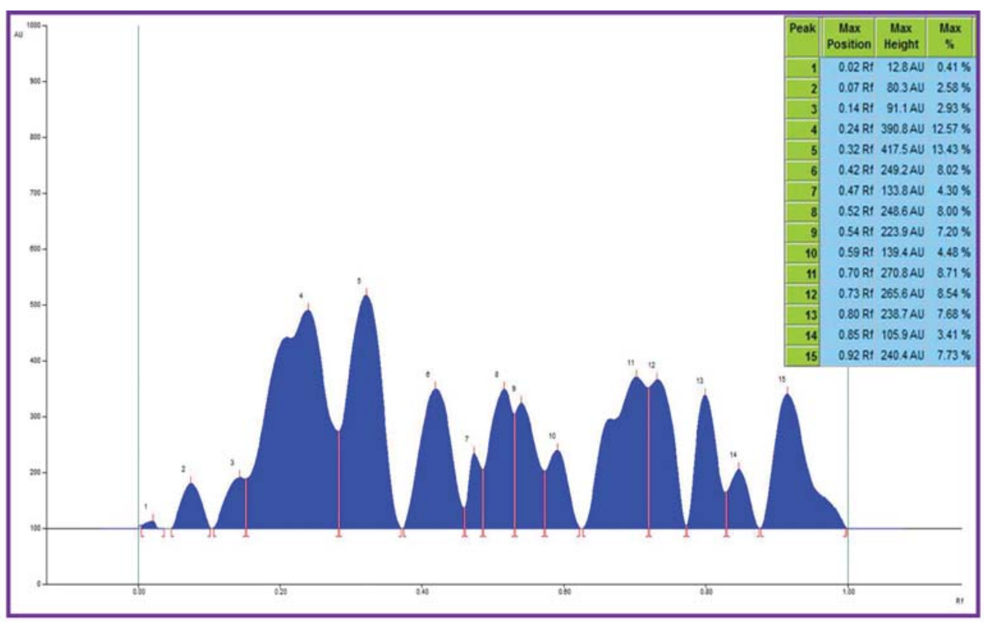


UV- 254 nm

UV- 366 nm

Visible Light (After derivatisation with vanillin – sulphuric acid reagent)

Fig. 1: TLC photos of chloroform extracts of three batch samples



Peak	Max Position	Max Height	Max %
1	0.02 Rf	12.8 AU	0.41 %
2	0.07 Rf	80.3 AU	2.58 %
3	0.14 Rf	91.1 AU	2.93 %
4	0.24 Rf	390.8 AU	12.57 %
5	0.32 Rf	417.5 AU	13.43 %
6	0.42 Rf	249.2 AU	8.02 %
7	0.47 Rf	133.8 AU	4.30 %
8	0.52 Rf	248.6 AU	8.00 %
9	0.54 Rf	223.9 AU	7.20 %
10	0.59 Rf	139.4 AU	4.48 %
11	0.70 Rf	270.8 AU	8.71 %
12	0.73 Rf	265.6 AU	8.54 %
13	0.80 Rf	238.7 AU	7.68 %
14	0.85 Rf	105.9 AU	3.41 %
15	0.92 Rf	240.4 AU	7.73 %

Fig. 2: HPTLC finger print profile of chloroform extract at 254 nm

TLC studies of alcohol extract

The TLC studies of alcohol extract are tabulated in Table - 4. All the three batch samples showed identical spot in UV-254 nm, UV-366 nm and visible light (after

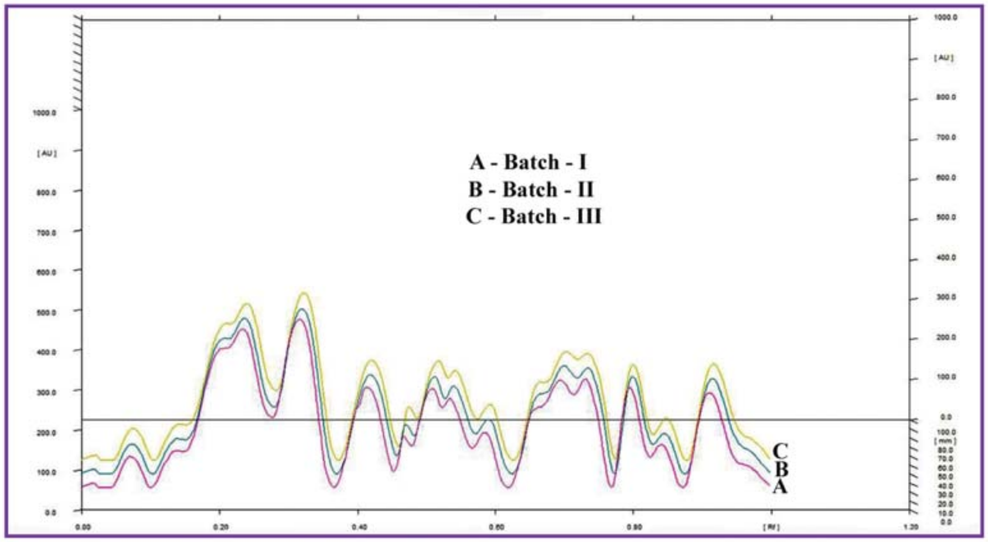
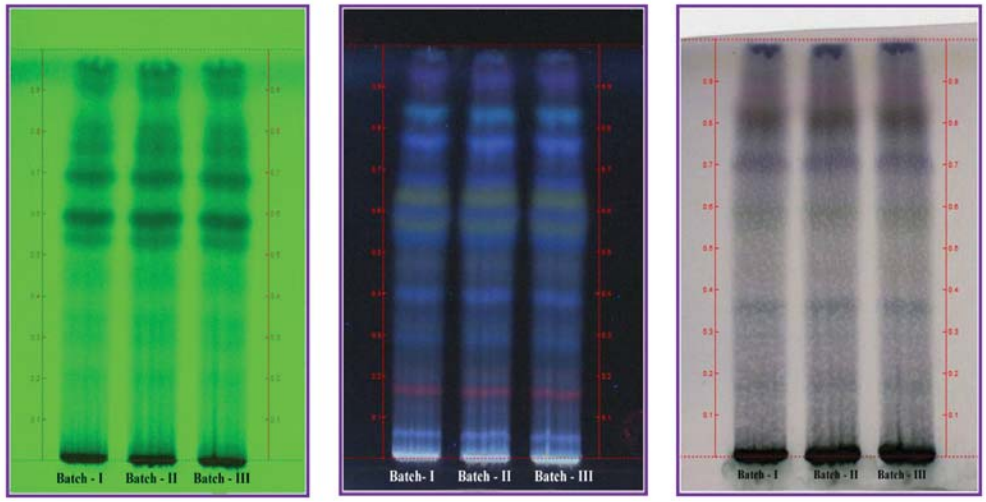


Fig. 3: HPTLC densitometry chromatogram of chloroform extracts of three batch samples at 254 nm

derivatised with vanillin – sulphuric acid reagent). In UV – 254 nm, 366 nm and visible light it shows 10, 13 and 7 spots respectively with different R_f values (Fig.4).

Table 4: R_f values of the alcohol extract

Solvent System		**R_f Values**	
	UV- 254 nm	**UV – 366 nm**	**Visible Light (After derivatisation with vanillin – sulphuric acid reagent)**
Toluene: Ethyl acetate (6:4)	0.90 Green	0.92 Fluorescent blue	0.87 Pink
	0.81 Green	0.83 Fluorescent blue	0.82 Grey
	0.76 Green	0.77 Fluorescent blue	0.71 Violet
	0.69 Green	0.67 Blue	0.58 Grey
	0.59 Green	0.63 Yellowish green	0.36 Blue
	0.53 Green	0.59 Blue	0.29 Grey
	0.44 Green	0.56 Yellowish green	0.18 Blue
	0.35 Green	0.53 Blue	
	0.28 Green	0.46 Blue	
	0.20 Green	0.39 Fluorescent blue	
		0.29 Blue	
		0.20 Brown	
		0.16 Red	



UV- 254 nm

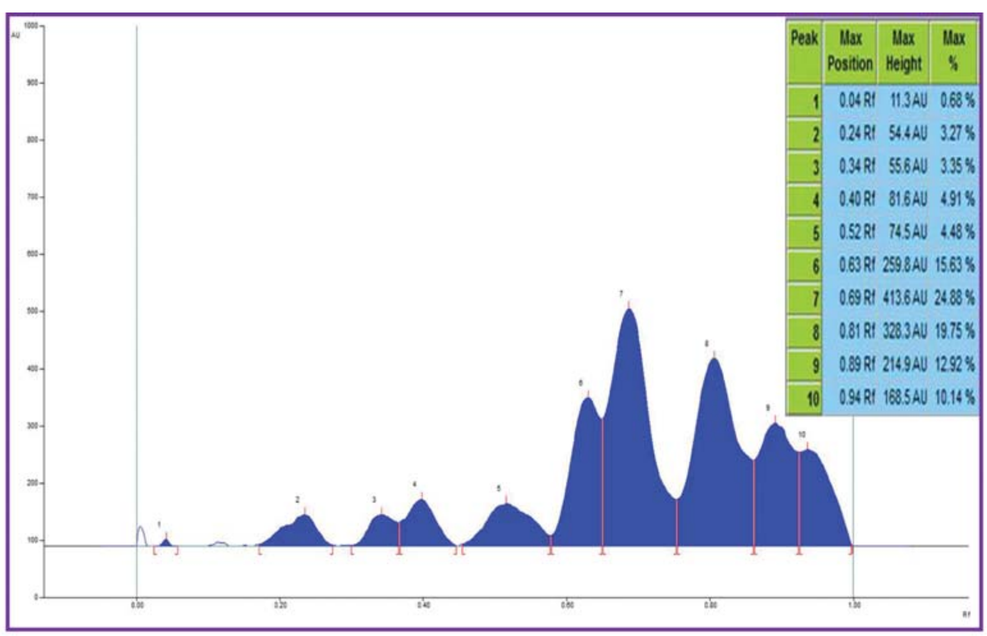
UV- 366 nm

Visible Light
(After derivatisation with vanillin – sulphuric acid reagent)

Fig. 4. TLC photos of alcohol extracts of three batch samples

HPTLC finger print studies of alcohol extract

The finger print of the chloroform extract shows 13 peaks of which peaks at R_f 0.63, 0.69, 0.81 and 0.89 were the major peak whereas peaks at R_f 0.04, 0.24, 0.34, 0.40, 0.52 and 0.94 were moderately smaller peaks (Fig. 5). The HPTLC densitometry chromatogram of alcohol extract of three batch samples were found to be same when scanned at 254 nm (Fig. 6).



Peak	Max Position	Max Height	Max %
1	0.04 Rf	11.3 AU	0.68 %
2	0.24 Rf	54.4 AU	3.27 %
3	0.34 Rf	55.6 AU	3.35 %
4	0.40 Rf	81.6 AU	4.91 %
5	0.52 Rf	74.5 AU	4.48 %
6	0.63 Rf	259.8 AU	15.63 %
7	0.69 Rf	413.6 AU	24.88 %
8	0.81 Rf	328.3 AU	19.75 %
9	0.89 Rf	214.9 AU	12.92 %
10	0.94 Rf	168.5 AU	10.14 %

Fig. 5: HPTLC finger print profile of alcohol extract at 254 nm

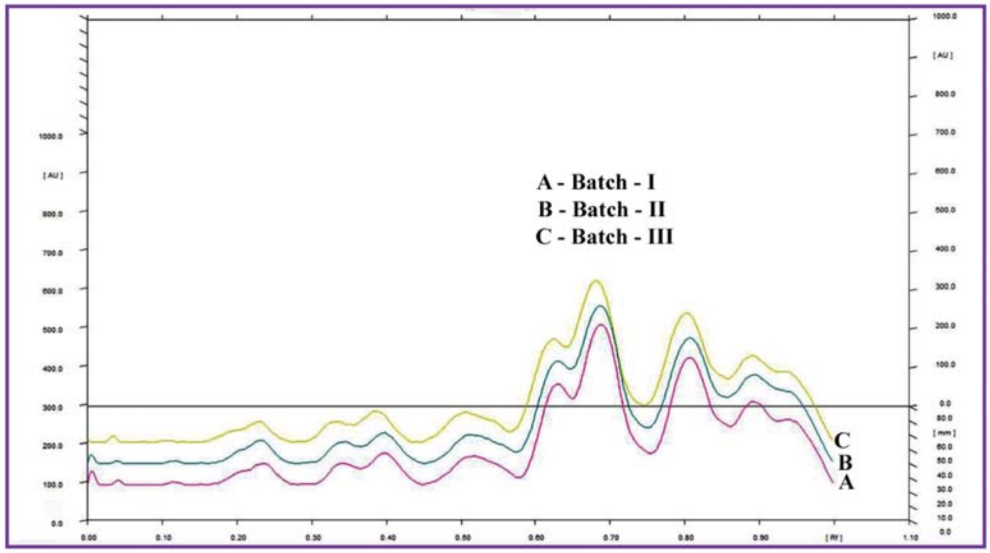


Fig. 6: HPTLC densitometry chromatogram of alcohol extracts of three batch samples at 254 nm

Microbial load, Heavy Metals, Aflatoxins and Pesticidal residues

Estimation of microbial load viz. Total bacterial count (TBC), Total fungal count (TFC), Enterobacteriaceae, *Escherichia coli*, *Salmonella* spp and *Staphylococcus aureus* were found to be within the permissible limit as stated by WHO (Table 5). The heavy metals viz. lead was present within the permissible limit where as cadmium; mercury and arsenic were not found in the drug (Table 6). The studies of other parameters like estimation of afltoxins such as B_1, B_2, G_1 and G_2 and pesticide residue such as organo chlorine group, organo phosphorus group, alachlor, aldrin, chlordane, DDT, endosulfan, heptachlor, lindane and malathion were not detected from the drug.

Table 5: Microbial Load

Parameters	**Results**	**WHO Limits for internal use**
Total Bacterial Count (TBC)	3 x 10^2 cfu/gram	$1x10^5$cfu/g
Total Fungal Count (TFC)	Less than 10 cfu/gram	$1x10^3$cfu/g
Enterobacteriaceae	Absent	$1x10^3$cfu/g
Escherichia coli	Absent	$1x10^1$cfu/g
Salmonella spp	Absent	Absent
Staphylococcus aureus	Absent	Absent

Table 6: Analysis of Heavy Metals

Sl.No	Parameters	Values
1.	Lead	0.0128 ppm
2.	Cadmium	Not detected
3.	Arsenic	Not detected
4.	Mercury	Not detected

All values are mean of three determinations

Conclusion

Standardization is an important aspect of any herbal formulation development. It is important to identify and record the physical, physicochemical and chemical properties of each plant material that is involved in product development of Majoon-e-Lana to maintain the batch-to-batch consistency and quality of the products. The physic-chemical parameters will be helpful for fixing pharmacopoeial standards of the drug. TLC/HPTLC finger print profile of chloroform and alcohol extracts provides a suitable method for monitoring the identity and purity and also standardization of the drug. Heavy metals, aflatoxins, pesticidal residues and microbial load were found to be within the permissible limit of WHO, indicating that the drug is free from toxic materials and which can be used in the ailments of stomachic, digestive and brain disorders.

Acknowledgement

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